The placement, fate and effectiveness of granular nematicides in potato beds infested with the potato cyst nematode *Globodera pallida* (stone)

Thesis

THE PLACEMENT, FATE AND EFFECTIVENESS OF GRANULAR NEMATICIDES IN POTATO BEDS INFESTED WITH THE POTATO CYST NEMATODE *GLOBODERA PALLIDA* (STONE)

Simon Roger Woods BSc (Hons)

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Collaborating Establishment:
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Rothamsted Experimental Station

Abstract

The chemical control of the potato cyst nematode (PCN) by granular nematicides when applied and incorporated into potato seed beds was investigated to assess problems connected with incorporation using bed cultivation machinery.

Fluorescent tracer granule work using a range of granular nematicide incorporation methods suggested that differences exist between the incorporation methods in terms of placement of the fluorescent granules in the planted potato bed. Incorporation of tracer initially by a bed tiller followed by a second incorporation by a stone and clod separator produced a distribution of tracer greater than 40cm deep in the planted bed. Incorporation of tracer by a stone and clod separator with application of tracer halfway up the first web produced concentrated bands of tracer in the sides of the planted bed. No visible differences in tracer distribution occurred between other treatments.

The differences observed between incorporation techniques during the fluorescent tracer granule work were shown not to be important in terms of PCN control or yield in the first year's field experiments. The second year of field experiments assessed the incorporation of the granular nematicide Vydate (10G) before, during or after stone and clod separation of potato beds. These field experiments suggested that timing of nematicide incorporation in relation to stone and clod separation had no effect on potato yield or control of PCN. As in the first year's experiments, significant differences occurred between plots treated or not treated with a granular nematicide, but not between incorporation methods.

Work describing the field concentration of oxamyl immediately after planting showed similarities to the distribution of tracer granules observed in the soil hall studies. The subsequent distribution of oxamyl 3 weeks after planting showed no redistribution of the nematicide in the potato bed. The depth of potato planting is thought to be responsible for the uniformity of PCN control and crop response to nematicide treatment regardless of incorporation method as seed was planted below the nematicide treated layer.

Evaluation of a diagnostic kit used for detecting oxamyl in soil showed that the kit was well suited for this purpose and its use is discussed in the light of the findings of this study.

Declaration

This thesis was composed by the author and is a record of work carried out by him on an original line of research.

All sources of information are shown in the texts and listed in the references; all help given by others is indicated in the acknowledgements.

None of this work has been presented in any previous application for a degree.

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Statement of advanced studies

During the tenure of this project, in addition to performing and reporting the experiments in this manuscript the author has also;

- Completed a course in statistical methods.

- Completed a programme of guided reading.

- Attended a number of seminars at Harper Adams College.

- Attended a number of conferences, namely:-

Offered Papers in Nematology, Rothamsted, December 1993.

ADAS Potato Conference, Peterborough, February, 1994.

Sampling to Make Decisions, Cambridge, March 1994.

Brighton Crop Protection Conference, November 1994.

Offered Papers in Nematology, Linnean Society London, December 1994.

The Management of Problems Caused by Plant Parasitic Nematodes, London, April 1995.

Integrated Crop Protection: Towards Sustainability? Edinburgh, September 1995.

Offered Papers in Nematology, Linnean Society London 1995.

Diagnostics in Plant Crop Protection, Warwick, April 1996.

PMB potato planting demonstration, Stafford, April 1996.

Third International Nematology Congress, Guadeloupe, July, 1996.

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1.0 CHAPTER 1.

INTRODUCTION TO POTATO CYST NEMATODE

1.1 THE POTATO CYST NEMATODES

1.1.1 Importance

The potato cyst nematodes (PCN) *Globodera rostochiensis* (Wollenweber,1923) Skarbilovich, 1959 and *G. pallida* Stone, 1973 are highly specialised plant parasitic nematodes. It is estimated that the reduction in potato production in the EU due to these two species is about 300 million ECUs per annum (Mulholland *et al.* 1996). A similar level of crop loss occurs in the UK, making PCN the most important group of pests on the approximately 141,000 ha of potatoes grown in the UK each year (Anon., 1995a).

Once established, a PCN infestation is practically impossible to eradicate and therefore the potato producer has to manage PCN infestations at a level which permits profitable potato production. This has placed great importance on quarantine of the pest in countries such as the USA to prevent its spread to uninfested areas.

1.1.2 Symptoms

A cycle of five potato crops may be necessary before field symptoms become visible (Jones and Kempton, 1982). The first symptoms of an infestation usually appear in July, when areas of maincrop potatoes may appear stunted and wilt readily in dry conditions. Later in the season the elliptical patches of stunted plants are likely to become invaded by weeds. Patches of fat hen, *Chenopodium album* may be indicative of PCN infestation. Examination of infested potato roots from late July to August will show white females (approx. 0.5mm diameter) on the root surface. The infested areas may senesce before the rest of the crop and show a reduction in yield.

1.2 Synonyms and History

The first report of the potato as a host to cyst-nematodes was in 1874, when Kühn suggested that *Heterodera schachtii,* the species name assigned to all cyst-nematodes at that time, was parasitising the crop. Between that time and the early 1900s the cyst-nematode of potato became more widely distributed throughout Europe and this led to the conclusion that one form of *H. schachtii* had specialised in using the potato as its host, causing "soil sickness of potatoes" (Spears, 1968). Wollenweber (1923) recognised morphological differences between the potato cyst-nematode (PCN) and the beet cyst-nematode, and proposed a separate species *Heterodera rostochiensis.* However, this species was not recognised until 1940, when Franklin produced a more complete morphological description and diagnosis of *H. rostochiensis*. In 1972 Stone recognised a second species of PCN, *H. pallida.* These "related " species were both within the subgenus, *Globodera,* defined earlier by Skarbilovich (1959) on the basis of the absence of a terminal cone on the posterior end of the female and the resultant round cysts. Subsequently Mulvey and Stone (1976) supported Behrens (1975) in elevating the round cyst-nematodes to generic rank with the result that *H. rostochiensis* and *H. pallida* became *Globodera rostochiensis* and *G. pallida*. Loof and Bakker (1992) have now obtained a ruling on the authorities for all the species within the genus *Globodera.* It has been decided to attribute the authors Wollenweber (1923) and Skarbilovich (1959) to *Globodera rostochiensis* and Stone (1973) to *Globodera pallida.*

The two species of PCN have been subdivided into pathotypes depending on their virulence towards seven differential *Solanum* clones. Kort *et al.* (1977) proposed a scheme for identifying pathotypes in both PCN species, five for *G. rostochiensis* and three for *G. pallida.* Nijboer and Parlevliet

(1990) re-analysed previously published data and concluded that only 3 recognisable Ro-pathotypes existed, Ro1/Ro4, Ro2/Ro3 and Ro5. They also concluded that *G. pallida* pathotype identification was unreliable because the differences in pathotype-specific virulence towards the different *Solanum* clones were too small to be significant. Genetic differences are known to exist within *G. pallida* with significant differences between three distinct European populations: Pa1, Luffness and Pa2/3 (Blok *et al.*, 1995). The need for a reliable pathotyping scheme, which relates the genetic diversity within the two species to corresponding resistance genes in *Solanum* clones, is important and will be discussed later in this chapter.

1.3 Distribution

PCN can be found in at least 58 countries around the world (Brodie, Evans and Franco, 1993). PCN originated in Andean regions of South America and were dispersed around the world along with their host, but probably did not arrive in Europe until the mid-nineteenth century when many potato lines were introduced in the search for resistance to potato blight. The nematodes were probably pests of pre-Inca agriculture, with *G. pallida* still present on *Solanum* in terraces uncultivated since ancient times (Jatala and Garzon,1987).

In the UK, PCN can be found in all the main potato growing areas with *Globodera rostochiensis* and *G. pallida* occurring in mixed or pure populations. However, a predominance of *G. pallida* in the most intensively cropped areas has recently been shown by ADAS soil sampling (Hancock,1988).

1.3.1 Distribution of PCN in fields

The distribution of nematodes or cysts in the soil could be random but as PCN cysts only move short distances with soil in a field as a result of cultivation practices it is more likely to be aggregated. In both cases (i.e. random and aggregated distributions) the variability of counts in soil samples (the ratio between some measure of dispersion and an estimate of the average number per sample) depends on this distribution. If the distribution of cysts were truly random then a single sample taken from anywhere in the field would suffice to give an estimate, with a known error, of the average density of cysts. However, the statistical errors inherent in taking and processing soil samples to estimate nematode populations have led to several different opinions on how PCN populations are distributed in the field. Jones (1955) based his sampling strategy on data approximating to a Poisson distribution, in which the variance is equal to the mean. This distribution type holds for counts of sub samples taken from a well-mixed bulk sample, but not for entire bulk samples.

The distribution of PCN in the field is typically patchy, except at high densities. Thus, Seinhorst (1982) found that a negative binomial distribution was a better fit than the Poisson distribution in field conditions.

Where data consist of counts of individuals their distribution can be described using Taylor's index of aggregation. This index relates the variance of counts of samples to some power of their means, and is often known as Taylor's Power Law (Taylor, 1984). More recently, researchers have adopted Taylor's Power Law to describe the distribution of PCN and to devise strategies for sampling them (Webster and Boag, 1992).

The patchy distribution of PCN in an infested field has a very plausible biological explanation. The nematodes probably arrive in the field in soil on dirty machinery and infected plant material and are deposited

irregularly (Brodie, 1993). They then spread from these initial foci as a result of agricultural operations and by water movement (i.e. flooding) to give an elliptical patch of infested soil with decreasing PCN density towards the edge of the patch.

1.3.2 Three dimensional distribution of PCN

A great deal of research has been carried out on the two dimensional distribution of PCN populations but the distribution of PCN populations in three dimensions has not been studied extensively. Whitehead (1977) found considerable variation in PCN population densities with depth. PCN were often as numerous at 20-40 cm depth as in the top 20 cm of soil but were uncommon below 40 cm. Brodie (1976) investigated the vertical distribution of several free living nematodes (*Belonolaimus longicaudatus*, *Pratylenchus brachyurus* and *Trichodorus christiei*) and found variation in populations with depth due to soil conditions such as temperature. The population dynamics of cyst nematodes and free living nematodes are different and hence there is a need for an assessment of vertical PCN distribution in relation to soil conditions and root characteristics. Such an investigation would allow any effects on population estimation techniques to be appraised (Haydock and Evans, 1994).

1.4 Biology and Life Cycle

The life cycle of PCN is typical of a cyst nematode (Fig.1). However, certain details of hatching, establishment of feeding, mating and cyst forming are unique to these nematodes, with differences occurring between the two species.

Cysts, often containing up to 600 eggs, can persist for up to 30 years where the soil type and climate are suitable (Grainger,1964). Hatching of the eggs can occur in water but, for substantial hatch to occur, stimulation by a hatching factor produced by potato roots is required.

These "potato root diffusates" produce a change in the permeability of the lipoprotein membranes of the eggshell thereby allowing an influx of solutes and oxygen to the egg (Atkinson and Ballantyne, 1977a, b).The effectiveness of the diffusates depends on temperature and, *in vitro* hatching of *G. pallida* generally occurs at lower temperatures than that of *G. rostochiensis* (Parrott and Berry, 1976). The rate of hatch *in vitro* is slower for *G. pallida* than *G. rostochiensis* (Evans, 1983) and differences in hatching activity *in vitro* of *G. pallida* occur between potato genotypes (Arntzen *et al.*, 1993). The hatching factors themselves are also thought to affect the two species differently. Twomey *et al.* (1993) associated the later hatch of *G. pallida* with potato root diffusates produced later in the growing season. They also found that *G. pallida* required a higher threshold level of hatching factors to induce hatch than *G. rostochiensis*. The different responses to soil temperature and different requirements in hatching factors may well result in little difference in the invasion characteristics of the two species with the two effects cancelling each other.

Hatched second stage juveniles (Plate 1) are attracted to enter the host at a root tip or the point of emergence of a lateral root. They move intercellularly through the root in the cortex, pericycle or endodermis, and eventually become sedentary, when they induce the formation of a large multi-nucleate syncytial transfer cell (Spears, 1968). The growing nematodes undergo three moults, firstly increasing in size to become third stage juveniles (J3) (Plate 2). Those with small syncytia generally become fourth stage vermiform males (J4m) (Plate 3) which emerge from the root

as the fifth (adult) stage; those with larger syncytia become fourth stage females (J4f) (Plate 4) and increase in size until the posterior end breaks through the root, becoming exposed for mating. Females produce a pheromone to attract fifth stage males (J5m) (Plate 5) for copulation (Green *et al.*, 1972). The fifth stage females (J5f) (Plate 6) enlarge as eggs inside the female's body develop. The female then dies and the female body becomes tanned as the cuticle hardens to produce the protective cyst.

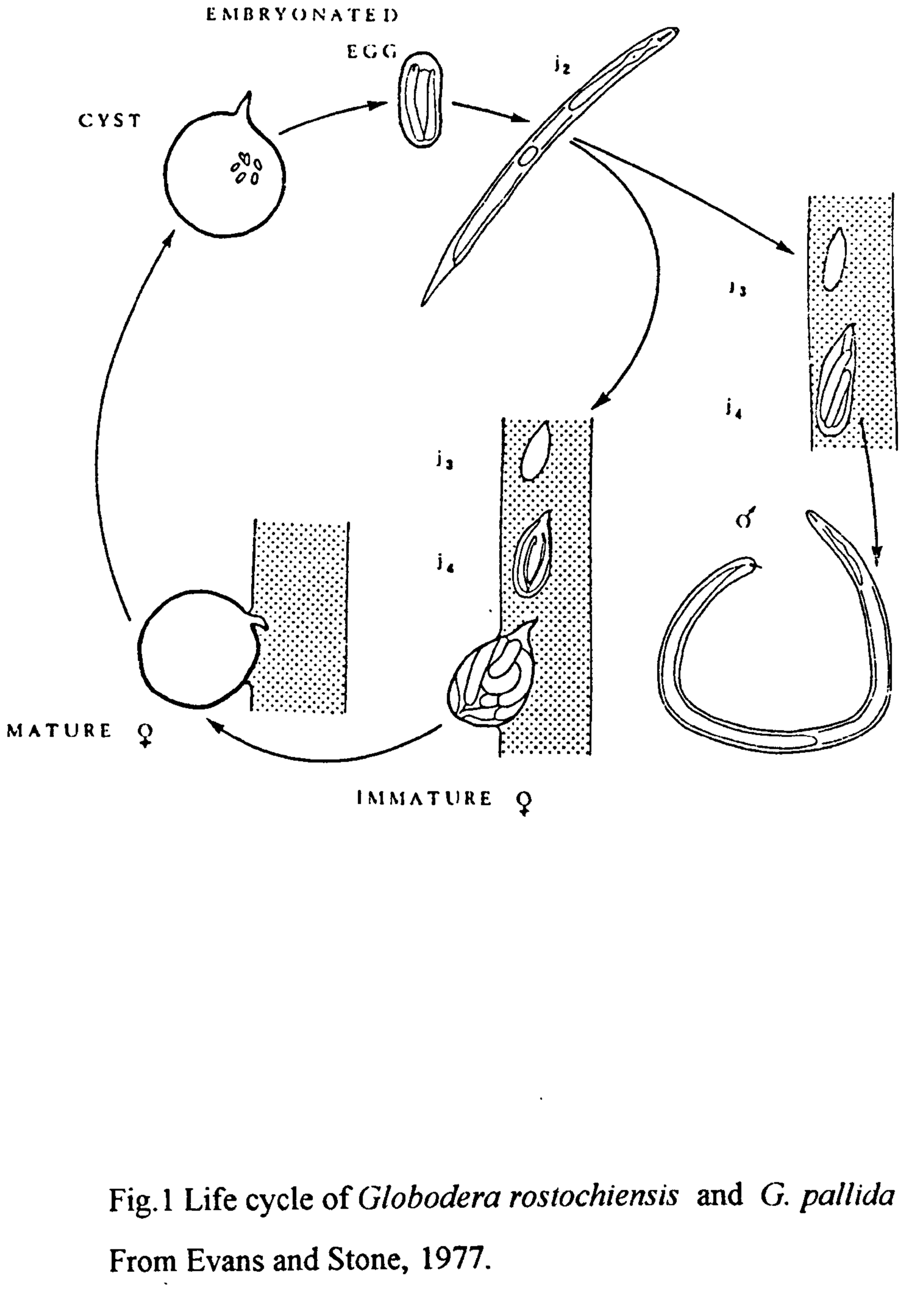


Fig.1 Life cycle of *Globodera rostochiensis* and *G. pallida*

From Evans and Stone, 1977.

Plates 1-5. Juvenile stages of the potato cyst nematode *Globodera pallida* as seen during root invasion assessment (x 100).

Plate 1. Second stage juvenile (J2).

Plate 2. Third stage juvenile (J3).

Plate 3. Fourth stage male juvenile (J4m).

Plate 4. Fourth stage female juvenile (J4).

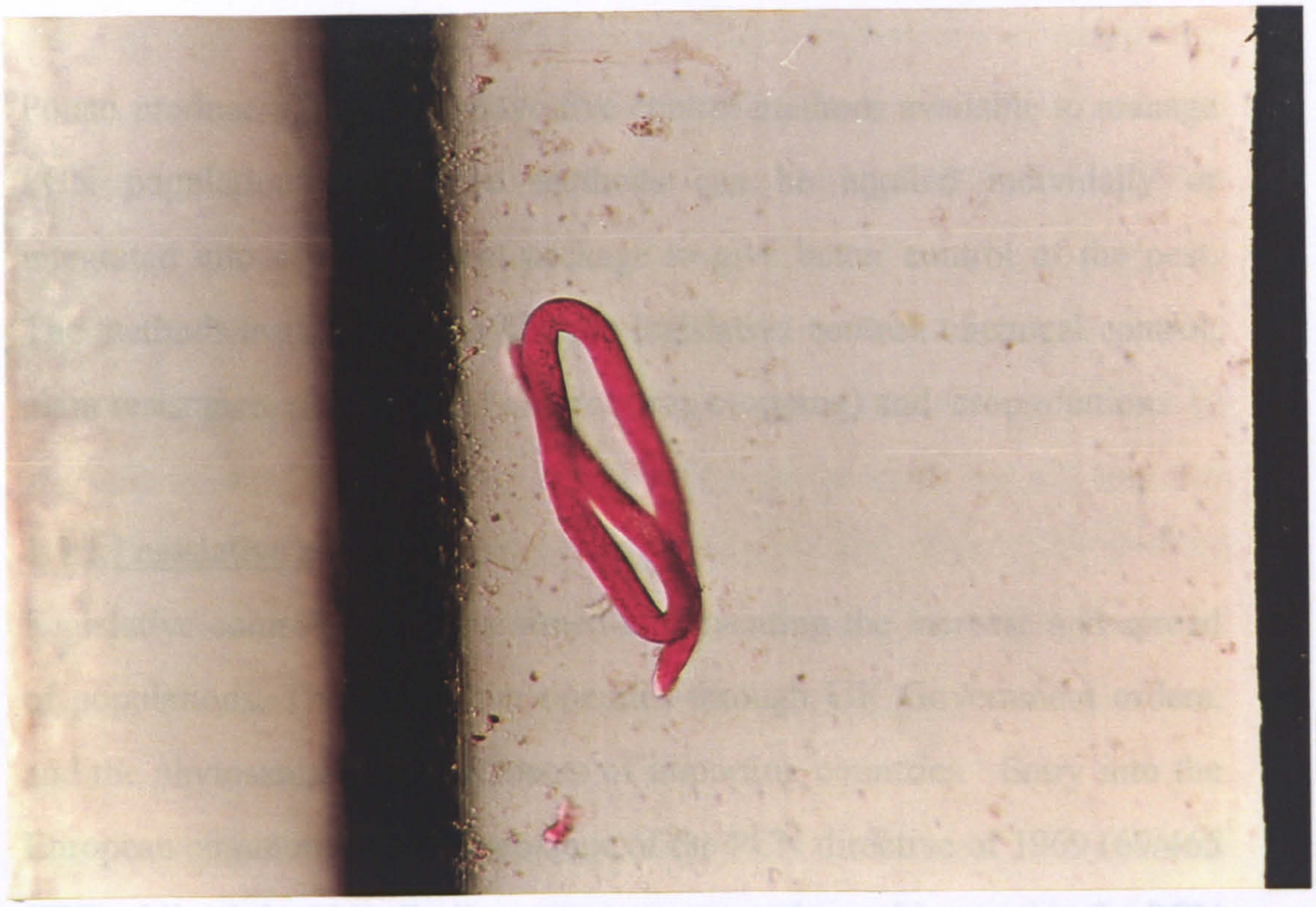


Plate 5. Fifth stage mature male (J5m).

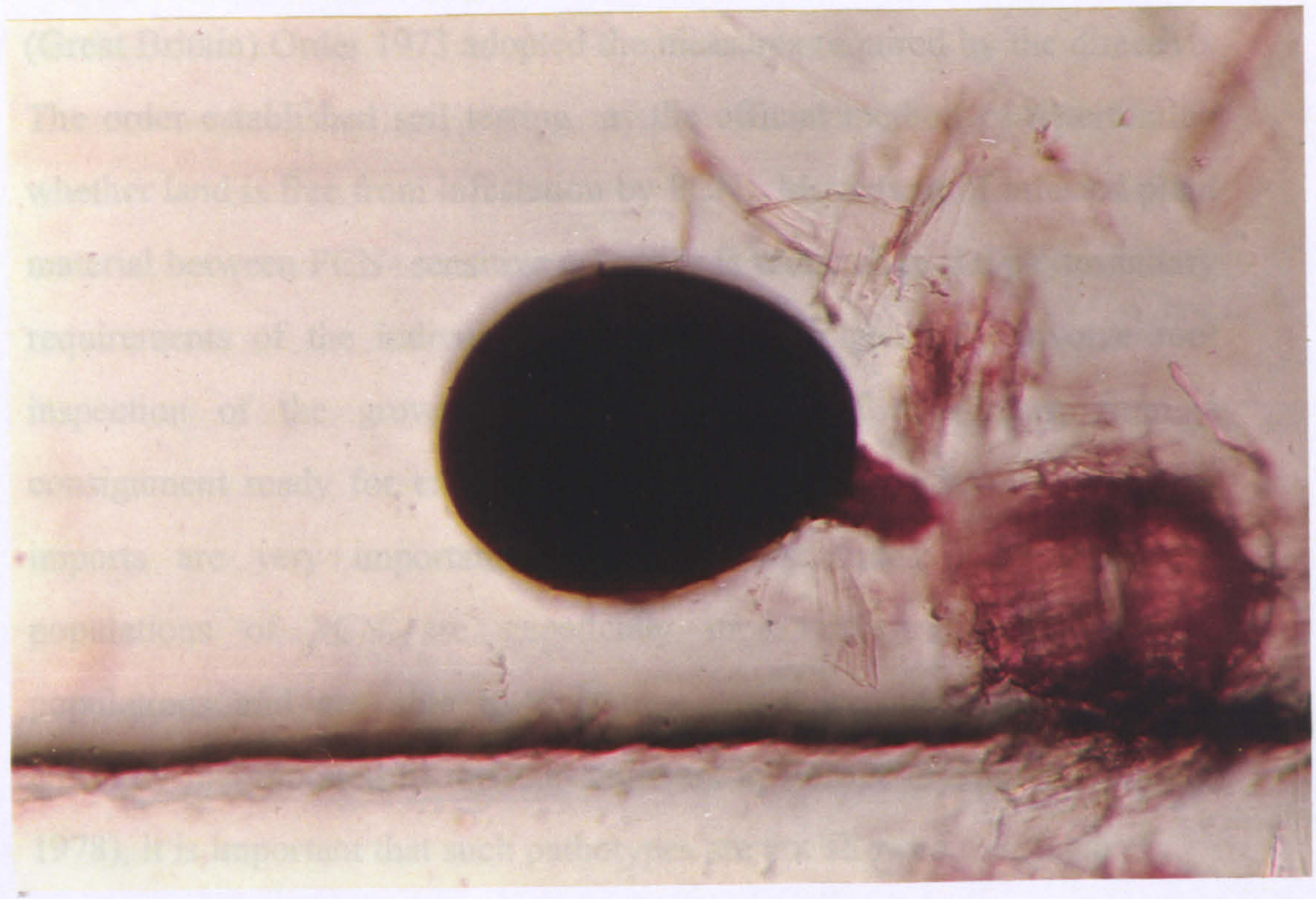


Plate 6. Fifth stage mature female (J5f).

1.5 Control of potato cyst-nematode

Potato producers in the UK have five control methods available to manage PCN populations and these methods can be applied individually or integrated into a management package to give better control of the pest. The methods available in the UK are legislative control, chemical control, plant resistance, phenological control (trap cropping) and crop rotation.

1.5.1 Legislative control

Legislative control of PCN is aimed at preventing the increase and spread of populations. The legislation operates through UK Government orders, and the phytosanitary requirements of importing countries. Entry into the European community and acceptance of the PCN directive of 1969 (69/465 EEC) obliged the U.K. Parliament to pass an order making testing for PCN mandatory on land which is to grow potato seed. The Potato Cyst Eelworm (Great Britain) Order 1973 adopted the measures required by the directive. The order established soil testing as the official method of determining whether land is free from infestation by PCN. Movement of infested plant material between PCN- sensitive countries is reduced by the phytosanitary requirements of the individual countries, which generally involve root inspection of the growing crop and testing of soil taken from a consignment ready for export (Anon., 1991). The regulations governing imports are very important because, for example, South American populations of PCN are genetically more diverse than European populations and are able to overcome resistance conferred by genes currently incorporated in resistant cultivars in Europe (Franco and Evans, 1978), it is important that such pathotypes are not allowed to spread.

1.5.2 Chemical Control

Two types of nematicide, fumigant and non-fumigant, are available for chemical control of PCN in the UK . Fumigant nematicides, such as 1,3-dichloropropene (Telone II) are used to kill eggs and juveniles inside cysts in the soil. The chemical is injected into the soil in the autumn and the soil surface sealed either by rolling or by polyethylene sheeting to prevent the volatile nematicide escaping. Covering the soil surface with polyethylene sheeting improved the efficacy of 1,3-dichloropropene but did not improve the efficacy of dazomet (Whitehead *et al.*, 1975b). The shift of PCN populations towards *G. pallida* has seen an increase in the use of fumigant nematicides such as Telone II because non-fumigant types work less well against this species (Crops, 1994).

Non-fumigant or granular nematicides represent the most widely used chemical control method for PCN in the UK. The oximecarbamates, oxamyl (Vydate 10G) and aldicarb (Temik 10G), are both applied to the seedbed prior to planting. The action of these chemicals is as acetylcholine esterase inhibitors, effectively paralysing the nematodes and preventing them from invading roots (Nelmes *et al.*, 1973). This action means they are sometimes refered to as nematostats. However, in this work they will be refered to as nematicides.

The fumigant nematicides do not require incorporation after injection into the soil, as they diffuse through the soil pores as gas. However, the non-fumigant granular nematicides require thorough incorporation into the top soil to be effective (Whitehead, 1975a). Poor incorporation of granular nematicides into potato beds has probably caused problems with nematicide effectiveness for PCN control. This will be discussed in Chapter 2.

Whitehead (1984) found that the control of PCN and the yield responses afforded by granular nematicides were dependent on the cultivar grown

and PCN species present, with tolerant cultivars showing less yield response to nematicides than intolerant cultivars. Differences in control of *G. rostochiensis* and *G. pallida* were also observed, with the former controlled better than the latter on a range of cultivars and soil types. However, these differences in control were not apparent in pot tests and Brown (1983) showed equally effective control of both PCN species by oxamyl or aldicarb in field tests. There is a great deal of variation in the published results of PCN control with granular nematicides. The reasons for this variation could lie in the nature of the pest's distribution in the soil or in variaitons between sites such as rainfall and soil temperature/type. The apparently poorer control of *G. pallida* in the field may be due to a range of factors such as the extended period of hatch resulting in invasion after the nematicide has degraded, or the variation in hatching efficiency of different cultivars.

1.5.3 Resistance and tolerance

Resistance to PCN in potato cultivars has been available since the 1960s. The host plant is not resistant in terms defined by standard plant pathology, as it is still invaded and damaged by juveniles and yield loss occurs. However, the capacity of the pest to reproduce successfully is reduced. Resistant cultivars limit the establishment of the feeding sites needed for female nematodes to develop, with the result that many juveniles become males. Many potato cultivars with single major gene (H1) resistance to *G. rostochiensis* pathotypes Ro1 and Ro4 are available. Single major gene resistance to *G. pallida* is not available but partial resistance to *G. pallida*, involving several minor genes, is available in commercial cultivars such as Santé. The control provided by such cultivars is variable depending on the virulence of the nematode populations to which they are exposed. Problems with the current pathotyping scheme used in the EC proposed by

Kort *et al.* (1977) means that many potentially useful lines with partial resistance to *G. pallida* are discarded by breeders because certain populations may increase slightly on them. The strict criterion of a Pf/Pi ratio ≤ 1 to class a potato clone as resistant to PCN is probably an obstacle rather than a help in breeding *G. pallida* resistant cultivars (Nijboer and Parlevliet, 1990). Trudgill (1985) concluded that before the problem of matching genetic variation within nematode species to specific resistance genes in potato can be solved, more reliable methods of measuring the genetic variation in the nematode and potato will be required.

Tolerance is the ability of a plant to withstand attack by a pest without suffering undue damage (Trudgill, 1986). Resistance and tolerance are independent (Fig. 2) but resistance may confer tolerance (Evans and Haydock, 1990). Tolerance exerts no selection pressure on the pest population but without resistance, tolerant cultivars may increase the population of the pest to levels where the tolerance is no longer able to cope with the pest burden.

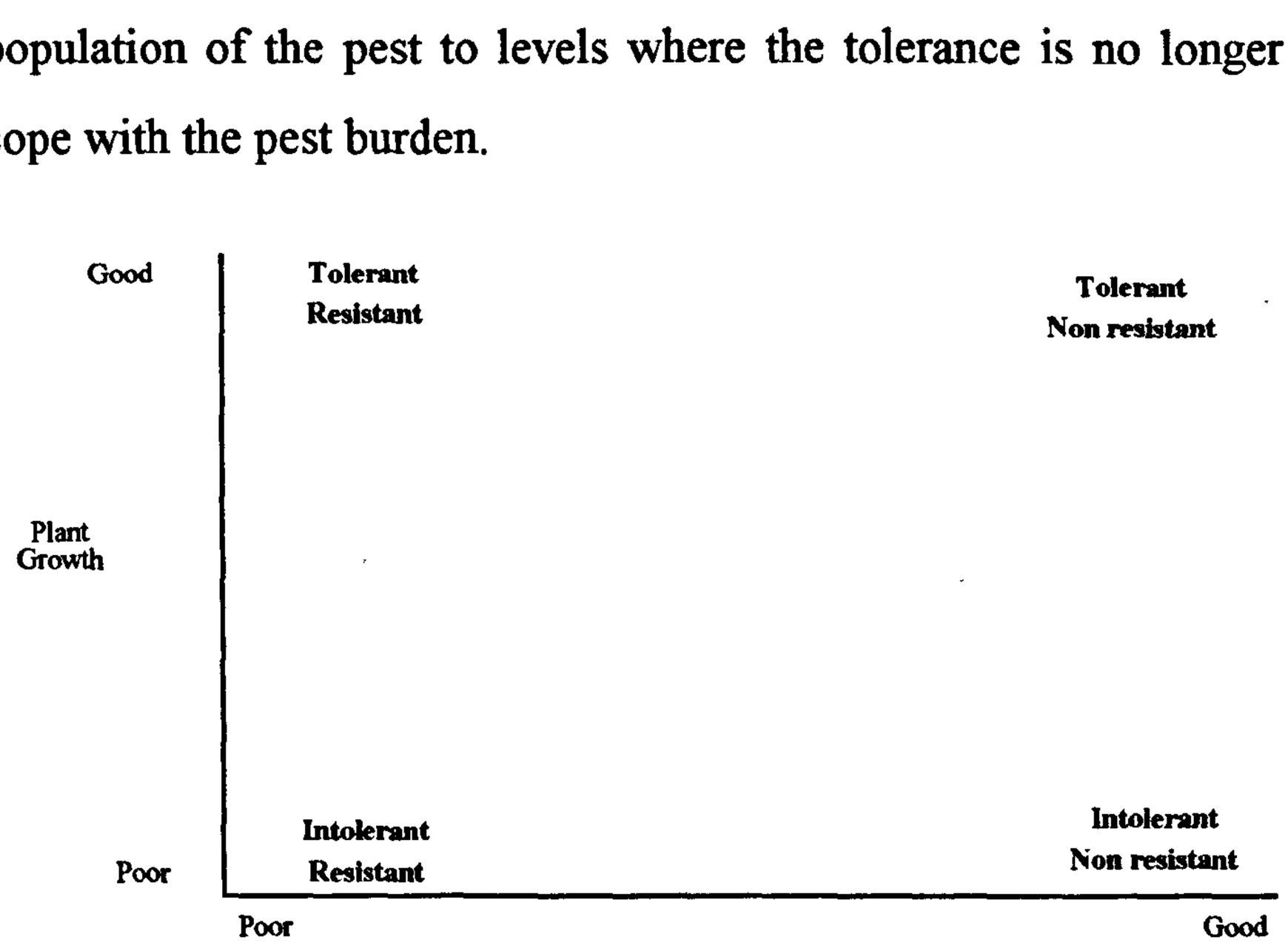


Fig.2 A diagrammatic representation of the independence of resistance and tolerance in plants, with all combinations of the two characteristics possible. (From Evans and Haydock, 1990).

The tolerant cultivars Cara and Maris Piper have become popular within the last 20 years. The widespread use of these cultivars is a contributory factor in the shift of PCN populations towards *G. pallida* as these cultivars are only resistant to Ro1 strains of *G. rostochiensis*. Figure 3 shows the areas grown of Cara and Maris Piper as a percentage of total maincrop potato production since 1973. A better classification of resistance and tolerance in commercial potato cultivars is required, such as scoring cultivars from 1 to 10 on these characteristics. However, the genotype x environment interaction that occurs in tolerance makes classification of this characteristic a long and expensive proposition as data has to be gathered from field trials.

1.5.4 Phenological control

Phenological control of PCN was assessed by Webley and Jones (1981). The effects of harvest date on population changes of both PCN species showed that harvesting 83 days after planting led to a decline in *G. pallida* populations and that *G. rostochiensis* populations declined when harvest occured up to 91 days after planting. Harvesting potatoes after these two dates caused populations of the respective species to fluctuate at about 120 eggs /g of soil. It would seem from this work that *G. pallida* can successfully reproduce in microplots one week faster than *G. rostochiensis*. Trap cropping or phenological control of PCN is a technique which has been used more in recent years for reducing infestations on heavily infested land. By growing a vigorous cultivar such as Cara, a substantial hatch of eggs is induced. The potato crop is then lifted and destroyed when maximum hatch and invasion has occurred, but before any new cysts have matured.

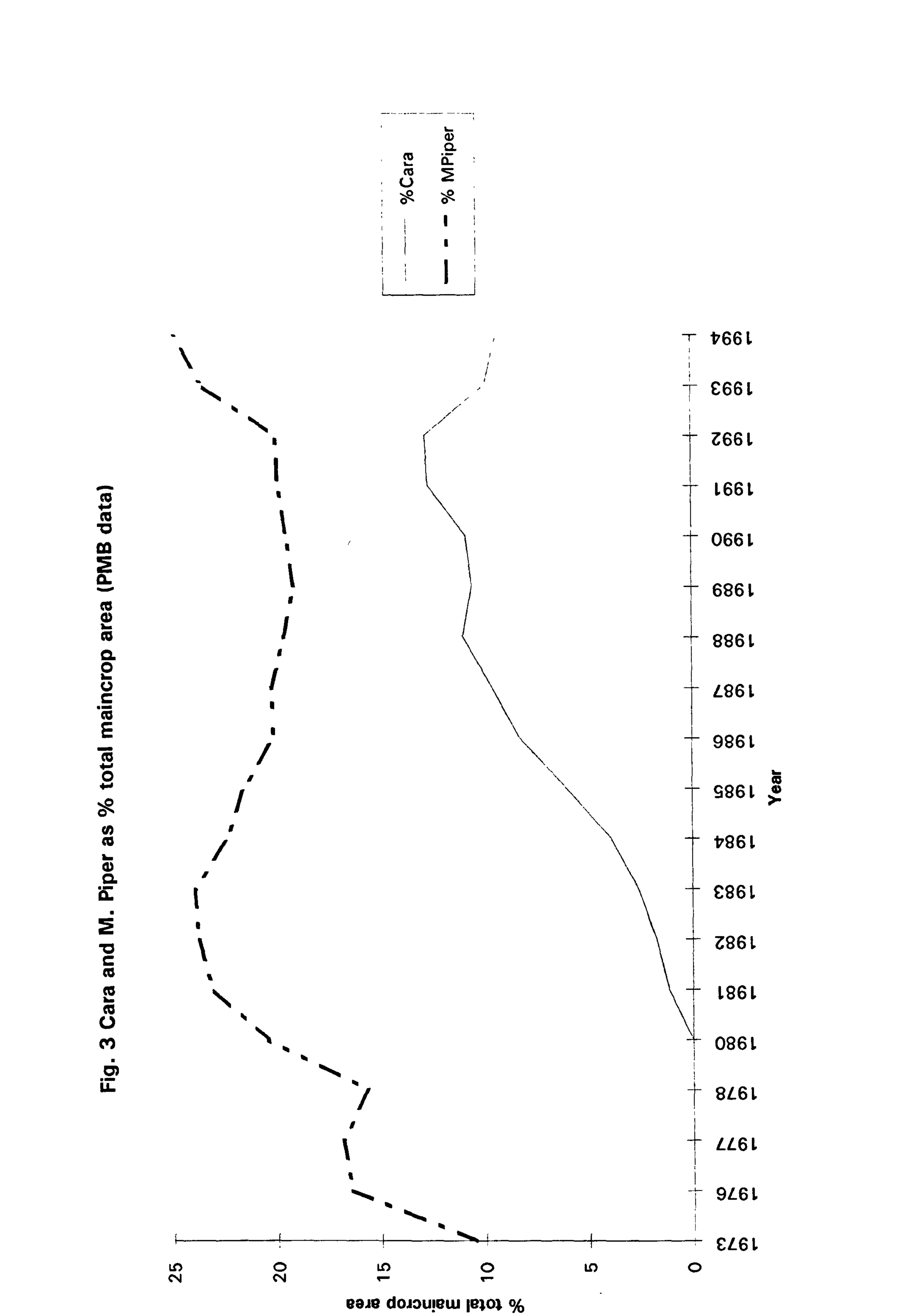


Fig. 3 Cara and M. Piper as % total maincrop area (PMB data)

This technique has proved very successful, with populations of 40-465 eggs/g of soil being reduced by 75% or more in six weeks (Whitehead, 1994a). However, if the crop is lifted too late a dramatic increase in PCN population density may occur, and it is unlikely that if left to maturity an economically valuable crop could be harvested. To increase the chance of lifting the trap crop at the correct time, nematode development in the roots could be estimated by monitoring soil temperatures (Webley and Jones, 1981), or observed directly by root examination.

With the introduction of set-aside in UK agriculture, the practice of trap cropping would seem a logical exploitation of the set-aside area in rotation. However, under current EC directives, the use of set-aside for trap cropping of PCN is prohibited.

1.5.5 Crop Rotation

Crop rotation is a vital part of any PCN management strategy. PCN requires a solanaceous host to reproduce with potatoes, tomatoes or aubergines the only significant host. Whitehead (1985a) found no weed species in the UK that could act as hosts for the pest. PCN declines naturally in the soil in the absence of a host as some eggs will hatch without the need for stimulation by potato root diffusate and some eggs will simply die. The decline rate is not entirely population density independent with large populations declining faster than small infestations on some soils (Whitehead *et al.*, 1980). An average decline rate of 33% is expected for *G. rostochiensis* in UK soils but the rate of decline for *G. pallida* is generally slower at ≅ 15% per annum (Whitehead, 1993). Such a rate of decline would require rotation cycles of 10 years or more if rotation was the only control tactic, and this is unacceptable for commercial growers. A crop rotation of 4-6 years, when used in conjunction with one or more of the other control methods, should result in

pre-planting nematode population densities that allow economic yields of potatoes to be achieved (Whitehead, 1986). However, since the introduction of effective non-fumigant (granular) nematicides in the 1960's and the concentration of potato production on the most suitable land, rotations have become shorter and 3 course rotations are now not uncommon. This means that there are nowsevere problems with PCN.

1.5.6 Integrated control

The term integrated control refers to the combined use of several of the control methods described above. The aim is to make the most efficient use of the control methods available and thereby maximise control of the pest. Jones (1969) demonstrated the effectiveness of combining several control methods for *G. rostochiensis*. Various combinations of rotation, resistant and susceptible cultivars, and nematicide were investigated. When a resistant cultivar was grown in nematicide treated ground in a 4 year rotation, a 99.9% kill of *G. rostochiensis* was observed over the cropping cycle.

Hancock (1994) conducted a trial to study the management of *G. pallida* populations in intensively cropped potato ground. Results indicated that the continuous production of potatoes on infested ground using a nematicide, soil fumigation and the partially resistant cultivar Santé was not economically viable or sustainable. Introducing a rotation of 1 in 2 improved the economic viability of the system but, to produce a sustainable cropping system, rotations of 1 in 4 would be required.

The integrated control of *G. pallida* has not been as successful as that for *G. rostochiensis* due to the lack of fully resistant cultivars and the apparently longer hatching period of *G. pallida*, which is believed to reduce the effectiveness of granular nematicides (Whitehead, 1992). The problem of few resistant cultivars is further compounded by the variability

in effectiveness of the partial resistance which can vary from field to field (Whitehead *et al.*, 1987).

1.5.7 Future developments

The future for PCN control looks promising. Research is under way to produce transgenic potato cultivars with resistance to PCN. One mechanism of resistance under consideration is that of causing potato plants to produce so called "plantibodies" which would bind to a specific part of the nematode such as the feeding apparatus (Ramos *et al.*, 1995). Other genetically modified crops have already been produced successfully, such as tobacco with resistance to tobacco mosaic virus and tomato with resistance to cucumovirus (Chen-ZhangLiang *et al.*, 1994).

New nematicides derived from fungi such as *Myrothecium* spp. may become available in the future. Trials with the biological nematicide Ditera® showed improvements in carrot yield and suppression of *Meloidogyne incognita* (Grau *et al.* 1996).

Other methods of PCN control that may be available in the future include the use of artificial hatching agents. These could be incorporated into the seedbed of a non-host crop to produce a mass hatch of PCN without chance for the pest to reproduce. However, a more successful approach would be to incorporate the genes for producing potato root diffusate into a non host crop genome such as wheat (Haydock P.P.J., *pers. comm.*).

Until the new methods of PCN control become available it is essential that the current methods of integrated control are improved. The integrated control of PCN is probably the best example of how successful the combination of several control strategies can be. However, it also shows that without sufficient knowledge of how the pest and crop interact, problems can be exaggerated, i.e. the widespread selection for *G. pallida* in UK ware areas.

1.6 Aims of the research

The aims of the research presented in the following chapters are as follows:

- Investigate different methods of incorporating granular nematicides into potato beds.

- Study the distribution of nematicide granules after incorporation and planting.

- Relate the distribution of nematicide granules to the position of the planted potato tuber.

- Study the effect of incorporation technique on yield of the potato crop and population dynamics of potato cyst nematodes.

- Monitor the distribution of the granular nematicide Vydate 10G (oxamyl) in field incorporation studies and the re-distribution of granules after planting.

- Examine the potential for making improvements in nematicide placement and incorporation.

- Make recommendations on the most suitable method of nematicide incorporation in beds for nematode control, crop performance and environmental impact of the nematicide.

2.0 CHAPTER 2

GRANULAR NEMATICIDES AND THEIR INCORPORATION INTO POTATO SEED BEDS.

2.1 THE GRANULAR NEMATICIDES

The granular nematicides most commonly used in potato production are the oximecarbamates aldicarb (Fig.4) and oxamyl (Fig.5).

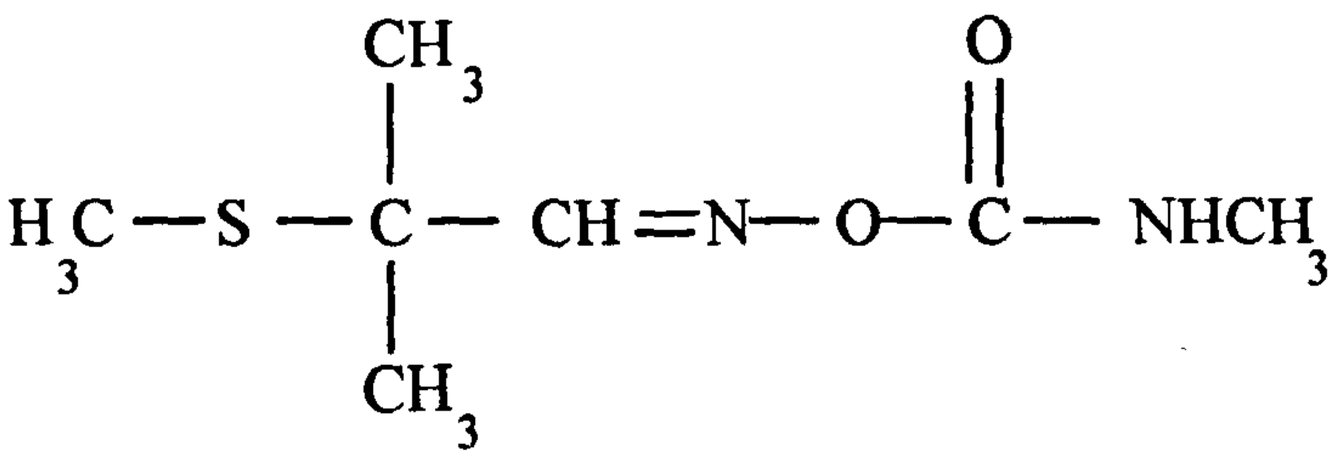


Figure 4. Structure of aldicarb. 2-Methyl-2-(methylthio) propionaldehyde *O*-(methylcarbamoyl) oxime

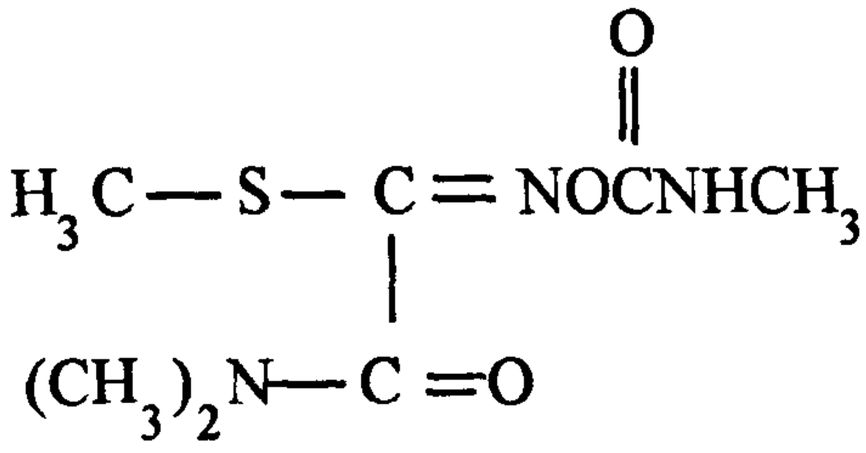


Figure 5. Structure of oxamyl. *S*-Methyl 1-(dimethyl carbamoyl)-*N*-[(methyl carbamoyl) oxy] thioformimidate.

The discovery that these compounds had nematicidal properties enabled formulations such as granules to be used, which gave effective nematode control at much lower dose rates than earlier fumigant nematicides such as methyl bromide.

The oximecarbamates act as nematostats, paralysing the nematodes present in soil water (Nelmes *et al.*, 1973). The mode of action is by acetyl-cholinesterase inhibition by carbamoylation of the enzyme's active site (Yu *et al.*, 1972). The oximecarbamates generally have high mammalian

toxicity, e.g. aldicarb has an LD_{50} (oral) to rats of approximately 1mg/kg (Cremlyn, 1978). Consequently aldicarb is only marketed in the UK as a dust-free granule (Temik 10G). Oxamyl has a lower mammalian toxicity with an LD_{50} (oral) to rats of 5.4mg/kg (Anon., 1994) and is used both as a granule and a liquid formulation (Vydate 10G).

The effectiveness of granular nematicides in a range of soil types is dependent on the polarity of the active ingredient. Non-polar compounds become adsorbed onto soil organic matter thereby reducing the concentration of the active ingredient in soil water (Bromilow, 1973). The activity of a nematicide against a nematode is also dependent on other factors such as the rate of breakdown in the soil, the rate of detoxification in the nematode and the activity against acetylcholinesterase. These factors are often difficult to predict and their effects are unknown for many compounds (Whitehead *et al.*, 1985b). Clearly further work is needed to determine the specificity of nematicides against individual nematode species.

2.2 Granular nematicide application

The application of granular nematicides for the control of plant parasitic nematodes varies according to the nematode species and the crop to be protected. Treatment of seed furrows with aldicarb is used with crops such as onions (Whitehead *et al.*, 1979a) and field beans (Hooper, 1984) to control the stem nematode *Ditylenchus dipsaci*. In the USA, Brodie (1983) found that soil applications of oxamyl at 5.6 kg a.i./ha in the seed furrow at planting provided good control of *G. rostochiensis*. However, Whitehead *et al.* (1973a) concluded that nematicide application to the furrow produced large, potentially phytotoxic concentrations of nematicide around the seed

tuber and only controlled nematodes in the immediate vicinity of the seed tubers.

Soaking potato tubers for 15 min in 8µg/ml aqueous solution of oxamyl before planting reduced the number of *G. rostochiensis* cysts that formed on potato roots but was phytotoxic. Five foliar oxamyl applications of 1.12 kg a.i/ha at weekly intervals after 90% plant emergence produced a PCN population decline, but was considered impractical due to the risk of high oxamyl residues in the harvested tubers (Brodie, 1983). Whitehead concluded that to control potato cyst nematode in the UK, granules should be broadcast on the soil surface and incorporated into the soil immediately before planting (Whitehead *et al.*, 1973a). This has now become the standard recommendation for granular nematicide application and incorporation (Anon., 1995b).

The granular nematicides are formulated as dust-free, non-sticky granules about 1mm in diameter. This formulation makes them safer to handle and apply to soil, thereby reducing the risk of operator contamination. Formulated as micro-granules, nematicides must be metered onto the ground at the correct rate and a number of methods have been used to achieve this. The use of chemical fertiliser spreaders was popular in the 1970's but has now been abandoned in favour of more accurate methods. Various electronic methods have been used but problems can arise with the tractor forward speed and the speed of the electric motor becoming desynchronised. This may have been responsible for the recent temporary withdrawal of aldicarb in some American states where possible over dosing of fields had occurred with subsequent leaching of the product into ground water (Knight, *pers comm.*). The most reliable way of metering granules is by using a ground-wheel driven method such as the Horstine Farmery Microband Applicator (Plate 7). This eliminates the risk of over-dosing

when the tractor is stationary, and dosing is generated by distance travellrd not the forward speed of the tractor.

2.3 Incorporation of granular nematicides before the use of potato beds

Non-fumigant granular nematicides do not actively permeate the soil and, although they are partly dispersed by soil water flux, must be mixed mechanically with the soil to protect the crop from nematode attack. Whitehead *et al.*, (1973a) observed that cyst nematodes can be controlled in field crops by the application of oximecarbamate or organophosphate pesticides to the seedbed at planting. The high cost of nematicides (e.g. £300 /ha) means that they must be used efficiently, in small quantities, and provide maximum benefit in terms of crop yield and nematode control. This is obtained by optimising the distribution of the chemical in soil in the area of early root development.

Row treatments may fail to control PCN and broadcast treatments with the granules subsequently mixed into the top-soil are generally recommended (Whitehead *et al.*, 1975a). Whitehead *et al.*, (1973a) observed that aldicarb and Nemacur controlled increase of potato cyst-nematode in a peat loam less well when incorporated with a rotary harrow than with an L-bladed rotavator. Subsequent tests (Whitehead *et al.*, 1975a) suggested that rotavators (L-bladed and spiked) mixed granules to the working depth of the implement, whereas harrows (spring-tine, reciprocating and rotary) gave a more shallow distribution. Smith and Bromilow (1977) observed that, in peat fen soils, aldicarb and oxamyl more reliably increased potato yields and controlled *G. rostochiensis* increase when incorporated by rotavators than by harrows. They attributed this to the deeper incorporation of granules achieved by rotavators. The optimum depth to which nematicides should be incorporated seems unclear. Whitehead *et al.*

(1975a) suggested that small amounts of granular nematicides might be more effective when incorporated shallowly (top 10cm) than deeply (top 20cm). Subsequent tests by Moss *et al.* (1976) showed that at 5.6 kg/ha oxamyl and aldicarb controlled both species of PCN better when incorporated to 15cm depth than to 7.5 cm. However, at 3.4 Kg/ha no difference between nematicide incorporation depths was found. The results from these trials also demonstrated the difficulty in conducting nematicide efficacy Field Expeiments where the aggregated distribution of PCN populations in the field can cause large variations in data which may be difficult to analyse statistically.

The use of rotavators for incorporating nematicides in some soils, such as clay and silt soils, may not be appropriate due to the risk of smearing and compacting the soil, causing an anaerobic seedbed in wet conditions. Peat soils may also be damaged by the intensive use of rotavators as the action of the machine tends to produce a "fluffy" seedbed which is prone to excessive drying and wind erosion. An alternative to rotavation as a method of incorporating nematicide granules is to apply the granules in vertical bands 10-15cm deep and then mix the bands laterally with a rotary harrow (Whitehead *et al.*, 1981). This technique was refined to decrease the damage to silt soil types by using closely spaced vertical bands 7.5cm apart of nematicide granules blown into the soil behind the tines of a Dutch harrow during seedbed preparation. This technique proved to be as effective at controlling PCN as broadcast application followed by rotavation (Whitehead, 1988).

There are well proven techniques for incorporating nematicides in the soil, i.e. rotavation or vertical band/rotary harrow. The effects of these methods on PCN control and crop yield are well documented (Whitehead *et al.*, 1981). However, the exact placement of the granules in the soil has not been studied in great detail. Approximate depth distributions have been

published (Bromilow *et al.*, 1979; Whitehead *et al.*, 1981) but these results are limited in that they do not show how the soil concentrations of a nematicide are distributed horizontally and vertically in the potato ridge.

2.4 The bed system of growing potatoes

The use of beds for growing potatoes has many advantages over traditional ridges. In ridge-grown potatoes, each row of potatoes is confined in a ridge. In a bed system, two or three rows of potatoes are confined to one bed which generally measures 72" (1.8m) wide. The increasing restrictions on water and nitrogen use plus tighter product specification has led producers, especially those on sandy soil types, to adopt beds due to the decreased surface area obtained when two ridges are combined to form one bed (Fig 6). Beds are suitable for coarse textured mineral soils due to the increased quantity of soil dug when the crop is harvested. This causes problems with harvest in heavy soil types. In wet conditions delays in harvesting can occur even on lighter soil types, but an advantage is that the extra quantity of soil passing over the harvester web may decrease tuber damage (Anon., 1986).

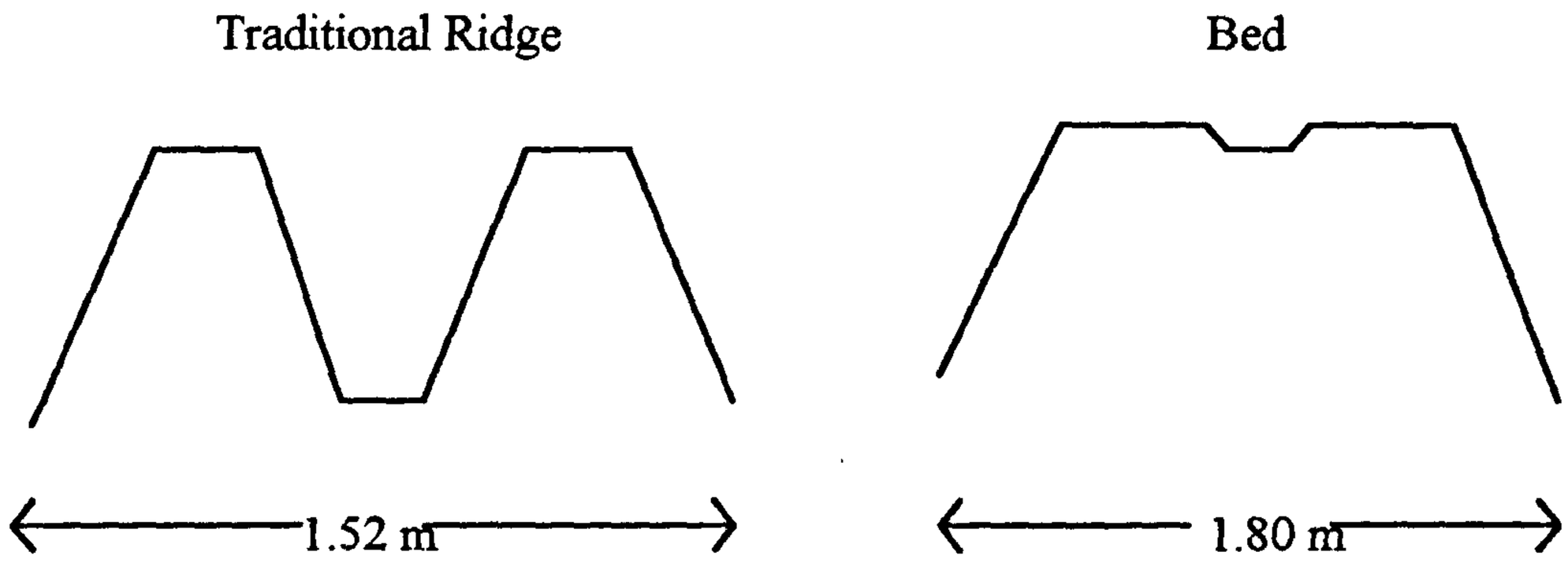


Fig. 6. Dimensions of a traditional ridged and a bed system for potato production.

2.5 Incorporation of granular nematicides into potato beds

Potato production on ground with a high stone content can often be less efficient as a result of stone damage to tubers at harvest, delays in harvest by stone blockages in harvesting equipment and the high costs of removing stones harvested along with the crop. To solve this problem, stone and clod separators (destoners) have become popular in such areas. These machines are essentially potato harvesters that remove stones and clods before planting in a potato bed, at the same time producing a fine seedbed tilth. The use of stone and clod separators for applying and mixing nematicides into the soil is a procedure adopted recently by many potato growers due to the savings in power, time and labour that occur in combining two operations. However, doubts have been raised over the efficacy of the machines for this purpose (Dr. T. Dawkins, *pers. comm.*). To date the results of only two trials have been published (Spaull and Tones,1986 a,b), in which the effect of nematicide incorporation during stone separation on PCN control and potato yield were investigated. These trials suggested that control of PCN by nematicides applied and incorporated using a destoner were comparable to that achieved by standard techniques i.e. broadcast and rotavation. However, these trials were not fully replicated, and a standard application / incorporation technique was not used for comparison. Many commercial trials have been conducted by nematicide manufacturers, but the results have not been published and are not widely known. Consequently, there is confusion amongst potato producers regarding which method of applying nematicides to potato beds is the most effective at reducing PCN increase and protecting yield. Data from trials by Rhône-Poulenc suggests that stone and clod separators do not mix the nematicide into the soil profile below 15cm providing that the application equipment is situated at the share /first web interface (Hancock, 1995). These trials also

suggest that using stone and clod separators is not as effective at reducing PCN increase as broadcast application followed by rotavation. This contradicts the results of Spaull and Tones (1986a,b) and also comments made by Whitehead (1994b), who suggested that these machines may over dilute a nematicide in the soil profile by incorporating them too deeply. There is a need for a thorough investigation of nematicide incorporation using stone and clod separators.

2.6 General materials and methods

2.6.1 Application and incorporation of nematicides

Nematicides, Vydate 10G (oxamyl) and Temik 10G (aldicarb), were applied and incorporated in the experiments described in subsequent chapters using a range of methods. All machines were set to the manufacturers' recommendations and used at the correct power take-off speeds. The machinery used in trials and experiments was as follows;

2.6.2 Spiked rotavator.

A spiked rotavator (Chapters 3, 4, 5 and 6) was custom fitted with a Horstine Farmery granule applicator system (Plate 7.) set up to apply oxamyl at the recommended rate (Plate 8). The nematicide was applied directly in front of the tines whilst the machine was in operation. The application of aldicarb in Field Experiment 1 (Chapter 4) was achieved by broadcasting the granules on the soil surface using a vertical band applicator (VBA) set in the broadcasting mode before incorporating the granules using the spiked rotavator.

2.6.3 Bed tiller

A bed tiller (Dowdswell Powavator) (Plate 9) (Chapters 3, 4, 5 and 6) consisted of a spiked rotavator with ridging bodies mounted behind the machine (A). This machine was fitted with Horstine Farmery granule applicators set to apply oxamyl at the recommended rate. The nematicide was applied directly in front of the tines whilst the machine was in operation. The application of aldicarb was achieved in the same way as for the spiked rotavator. To enable the VBA to broadcast the granules onto the surface of the bed, the appropriate delivery outlets were blocked off on the machine to give the desired working width. After the bed tiller a Grimme Colt de-stoner (Plate 11) was used to destone the plot and this gave further incorporation of the nematicide for some treatments.

2.6.4 Stone and clod separator

Three models of destoners were used during the research. In Field Experiment 2 (Chapter 4) a Pearson Megastar (Plate 10) was used to apply and incorporate the granular nematicides. This type of machine uses banks of rotating stars to remove stones. The Megastar was fitted with a set of Horstine Farmery Microband Applicators which were modified to apply both Temik and Vydate at the recommended rates. The fish tails of the application equipment were situated halfway up the first bank of stars on the machine. This is contrary to the current recommendations given by Horstine Farmery and both nematicide manufacturers' literature but when this machine was made this information was not available. Field Experiments 1, 3 (Chapters 4 and 5) and the fluorescent tracer experiment (Chapter 3) used a Grimme Colt custom-fitted with two sets of Horstine Farmery applicators; one set to apply oxamyl at the recommended rate (A) and the other to apply aldicarb at the recommended rate (B) (Plate 11). Both applicators fed into the same set of fishtails, which were situated

directly over the share/first web interface. The third destoner used was a Grimme Mustang (Plate 12). This machine applied and incorporated the nematicide Vydate to Field Experiment 4 (Chapter 5). The applicator and fish tails were again situated over the share first web interface. Both the Grimme Colt and the Mustang use traditional webs to separate stones and soil. They also incorporate a heavy top scrubber web which sits above the lower rotating webs and helps to break up clods as they pass between the two webs.

2.6.5 Vertical band applicator

A vertical band applicator (Plate 13) as described by Whitehead *et al.* (1981) was used to apply oxamyl and aldicarb to plots at recommended rates in Field Experiment 1 (Chapter 4). The working width of the machine had to be reduced to fit in with the beds used in the experiment. This was achieved by blocking off some of the outlets on the machine. A Roterra was used to achieve lateral mixing of the vertical bands of nematicide produced by the VBA.

Plate 7. Horstine Farmery Microband Applicator.

Plate 8. Spiked rotavator fitted with Horstine Farmery granule applicators.

Plate 9. Bed Tiller.

Plate 10. Pearson Megastar stone and clod separator. Plate courtesy R. Pearson Ltd.

Plate 11. Grimme Colt stone and clod separator.

Plate 12. Grimme Mustang stone and clod separator. Plate courtesy of R. Pearson Ltd.

Plate 13. Vertical Band Applicator.

3.0 CHAPTER 3

THE DISTRIBUTION OF FLUORESCENT TRACER GRANULES IN THE SOIL PROFILE USING DIFFERENT INCORPORATION MACHINERY.

3.1 Introduction

A knowledge of how cultivation practices move soil or particles added to soil, such as fertiliser granules, lime or granular nematicides, is necessary if improvements are to be made to farming practices. Choice of cultivation technique affects the depth to which soil is disturbed and also how deeply and thoroughly a soil ammendment is incorporated. This has consequences for the efficacy of fertiliser and chemical amendments, and for environmental concerns in areas of high water tables where overly deep incorporation may lead to groundwater contamination. This is particularly so in relation to the methods of granular nematicide incorporation presently adopted by potato growers in the UK. The stone and clod separator may be responsible for nematicide distributions which will not provide effective PCN control in the early potato rooting zones. However, no data has been published which provides evidence to support the theory of "banding" or "over dilution" mentioned in Chapter 2. Therefore a method is needed to visually assess the placement of nematicide granules in the soil after they have been incorporated by potato cultivation equipment to see if different equipment and procedures result in different distributions.

To monitor soil amendment movement, a tracer substance which clearly marks the area under investigation and some way of assessing and recording the movement of the tracer is required. Several methods have been developed to do this. Studies using iron filings and fluorescent dyes as tracer materials have been conducted. In both cases soil cores were taken and divided into depth fractions. The weight of iron filings recovered from the samples and counts of fluorescent dye particles were used to give an indication of where and how much of a chemical was in the soil (Staniland, 1959).

James and Wilkins (1965) and Thompson *et al.* (1981) used fluorescent dyes incorporated into the soil surface to determine the incorporation characteristics of several cultivation practices. Visual assessment of soil profile photographs taken at night using U.V. light provided qualitative information on the performance of the machinery used.

A similar method was adopted by Barrentine *et al.* (1965), Read *et al.* (1968) and Lalor and Smith (1973), where the amount of fluorescent dyes present in soil depth fractions was determined by fluorometry or gas chromatography. A quantitative assessment of incorporation was achieved but the processes involved were both time consuming and expensive.

Radioisotope tracers can be employed to measure incorporation methods quantitatively (James and Wilkins, 1965). However, this technique requires specialised equipment and a licenced site on which to conduct trials.

Collier *et al.* (1981) descibed a quantitative photographic technique for the analysis of the incorporation of soil applied chemicals. Emitted light intensity from fluorescent dye incorporated into soil samples by cultivation equipment was compared with samples of known dye concentrations. However, tracer calibration of different soil types and moisture contents was required.

Sallyani and Bowen (1983) used a computerised digitising tablet to obtain quantitaive data on soil ammendment incorporation. By depressing a pen like sylus on a speck of fluorescing tracer material on a photograph a co-ordinate could be generated for the tracer position in the soil profile. However, they noted that problems arose in distingusihing between tracer particles and flakes of dye which had become detached from the sand particles used as a base for the dye.

The main requirement for this research is to obtain an initial indication of which incorporation methods currently adopted by UK potato growers are likely to decrease the efficacy of a granular nematicide. The work

mentioned above falls into two areas, qualitative and quantitative investigation. Quantification of the results, whilst useful, may not be necessary in the early stages of this research. The aim of this piece of work is to decide the incorporation methods on which to concentrate in later field and laboratory studies, so qualitative results alone are sufficient. To appraise the distribution of granular nematicides when incorporated using a stone and clod separator as compared with other potato ground cultivation machinery, the following method was devised.

3.2 Materials and Methods

3.2.1 Preparation of fluorescent tracer granules

A batch of sepiolite, the base material for Vydate (10G), was dry dressed with the fluorescent pigment Radglow (Ciba Pigments, Hully Road, Maccelesfield, Cheshire) at a rate of 2.5 % w/w (i.e 5g pigment to 200g sepiolite) (Dr R. Robinson *pers comm*). This rate of pigment amendment provided satisfactory fluorescence of the granules in the soil. The pigment chosen is the same as that used to coat sugar beet pellets and is highly visible in both visible and ultra violet light. The base material was dry dressed with the pigment by shaking 200g of the sepiolite with 5g of the pigment in a 500ml plastic beaker for 5 minutes. This rate gave sufficient granule fluorescence and minimised the danger of surplus pigment coating the soil as well as the granules. It should be noted that the flow rate of these tracer granules and their characteristics in the soil profile will be very close, but may not necessarilly be identical to those of the product Vydate.

3.2.2 Construction of light proof tent

A light proof tent was required to enable photographs of the tracer granules to be taken in pure ultra violet light. The tent was constructed from black plastic silage sheeting (8m x 4m). The sheeting was supported by flexible glass fibre canes bent into hoops which when erected produced a tunnel 6m long and 2m diameter (Plate 14). When the base of the tent was covered with soil, visible light was excluded adequately from the inside of the tent.

3.2.3 Application and incorporation of tracer granules

Tracer granules were applied at 110Kg/ha using micro band applicators (Horstine Farmery, North Newbald, York) mounted on the incorporation machinery. This rate is the maximum output that can be gained from 14mm rotors in the microband hoppers used. The rate of application is twice the maximum recommended rate for Vydate (10G) (55Kg/ha). This rate was used to gain the maximum possible number of granules in the soil profile so that the location of the tracer granules was clear. At 55Kg/ha it was difficult to locate sufficient tracer in the profile for photography. The experiment took place in the soil hall at Harper Adams College. This facility is a covered field which allows the use of field scale operations in controlled soil and environment conditions. The soil type in the soil hall is a sandy clay loam of a similar type used in later field experimentation. Five application / incorporation treatments were used as follows;

- Dowdswell Powavator bed former applying and incorporating tracer granules to the bed.

- Bed former applying and incorporating tracer granules to the bed followed by a Grimme Colt stone and clod separator giving a second incorporation of the granules.

- Stone and clod separator (Grimme Colt) applying and incorporating the tracer granules with the applicators mounted at the share / first web interface.

- Stone and clod separator (Grimme Colt) applying and incorporating the tracer granules with the applicators mounted half way up the first web.

- Vertical band applicator for the application of tracer granules, followed by lateral incorporation of the granules using a Roterra.

Three replicate profiles were excavated and photographed for each treatment. After photography of the unplanted beds, a potato planter with ridging bodies mounted behind it was passed through each treatment bed producing two rows of potatoes. Three replicate profiles were then excavated in each planted bed. All plots were 10m long and 1.8m wide. This allowed three profiles to be excavated before and after planting.

3.2.4 Preparation of soil profile and illumination using ultraviolet light

Three soil profiles were excavated consecutively by making near vertical cuts across each treatment bed using a spade. Soil was removed to a distance 1.5m from the face. The face had to be cut slightly off the vertical in order to prevent collapse of the profile. After the initial excavation a first 2m long U.V light source (Slyvania Black Light-Blue F36W-BLB) was positioned 40cm above the profile and a second at the base of the profile (A,B) (Plate 15). The light proof tent (C) was placed in position and

the face illuminated by the UV tubes. Further minor excavation of the face was carried out using the blade of a pen knife. Starting at the top of the profile, soil was gently picked away to reveal an undisturbed face. This gave maximum clarity and ensured that the position of the granules was that which had been caused by the incorporation method, and not by subsidence or smearing of the profile during the initial excavation. A 2.5m length of white rope (D) was placed over the top of the profile in order to show the outline of the bed, and a depth marker with 5cm increments drawn onto it was placed against the profile during photography to indicate the depth to which the tracer had been incorporated. This procedure was then repeated twice at 0.5m intervals into the bed to produce replicated results.

Plate 14. Light proof tent erected over potato bed in the soil hall.

Plate 15. Location of UV light sources above and below a soil profile.

3.2.5 Photography

3.2.5.1 Initial approaches

Initially photography was carried out using a Nikon F17 camera fitted with a Sigma F= 28-70mm (1:3.5 ~ 4.5) wide angle zoom lens using 35mm Fujicolour print film with a film speed of 400 ASA. A range of exposure times and filters were used (Table 1.).

Table 1. Camera and filter setting during initial photography.

Filter Type	Aperture Setting	Exposure Time (seconds)
No filter	f8	Auto
No filter	f8	+3
No filter	f8	+5
No filter	f11	Auto
No filter	f11	+3
No filter	f11	+5
Polarising Filter	f8	Auto
Polarising Filter	f8	+3
Polarising Filter	f8	+5
Polarising Filter	f11	Auto
Polarising Filter	f11	+3
Polarising Filter	f11	+5
Orange Filter	f8	Auto
Orange Filter	f8	+3
Orange Filter	f8	+5
Orange Filter	f11	Auto
Orange Filter	f11	+3
Orange Filter	f11	+5
Orange + Polarising	f8	Auto
Orange + Polarising	f8	+3
Orange + Polarising	f8	+5
Orange + Polarising	f11	Auto
Orange + Polarising	f11	+3
Orange + Polarising	f11	+5

3.2.5.2 Final Photography

It was found that the best results were achieved using 35mm Fuji colour print film with a film speed of 400ASA. A Nikon F17 camera with a Sigma F= 28-70mm (1:3.5 ~ 4.5) wide angle zoom lens fitted with an orange filter was used to take the photographs. The camera was mounted on a small (40cm high) tripod 1.4m away from the profile to provide stability during the long exposure time of 60 seconds at f8 aperture. For close ups the same camera settings were used, but the camera was moved to a position 70cm from the profile.

3.2.6 Image analysis of photographs

Image analysis of the photographs was investigated using Cyclops v 2.31 image analysis software. However, the ability of the computer programme to distinguish between the tracer granules and other soil materials such as organic debris was very poor, and I was not confident in the results that the computer generated. Therefore, results will be presented as the photographs obtained from the experiment.

3.3 Results and Discussion

3.3.1 Initial photography

Plate 16 shows the image obtained from a soil profile after photography using no filters with an aperture setting at f8 and a +5 exposure time. From the plate it is apparent that the UV light sources used are producing a diffused light to which the film is sensitive and which is probably interfering with the visible light emitted from the fluorescing tracer granules in the soil profile. This was unsatisfactory as the tracer is poorly defined as the barely visible light coloured specks in the photograph.

Plate 17 shows an image of a soil profile taken using a polarising filter with an aperture setting at f8 and an exposure time of +5. The polarising filter was used in an attempt to sharpen the image and to combat the diffuse light coming from the UV light sources. The image obtained was improved, more detail was visible and the tracer granules could be seen as orange specks in the profile. However, the contrast between the soil and the tracer is poor. The UV tubes emitted visible blue light as well as UV which interfered with the visible orange light produced by the tracer granules. In order to prevent blue light entering the camera lens, an orange filter was used (Plate 18.). This greatly enhanced the visibility of the orange light emitted by the tracer granules and made the tracer granules clearly distinguishable in the profile. This camera set up was considered to be the most suitable and was used to record results of all the incorporation experiments.

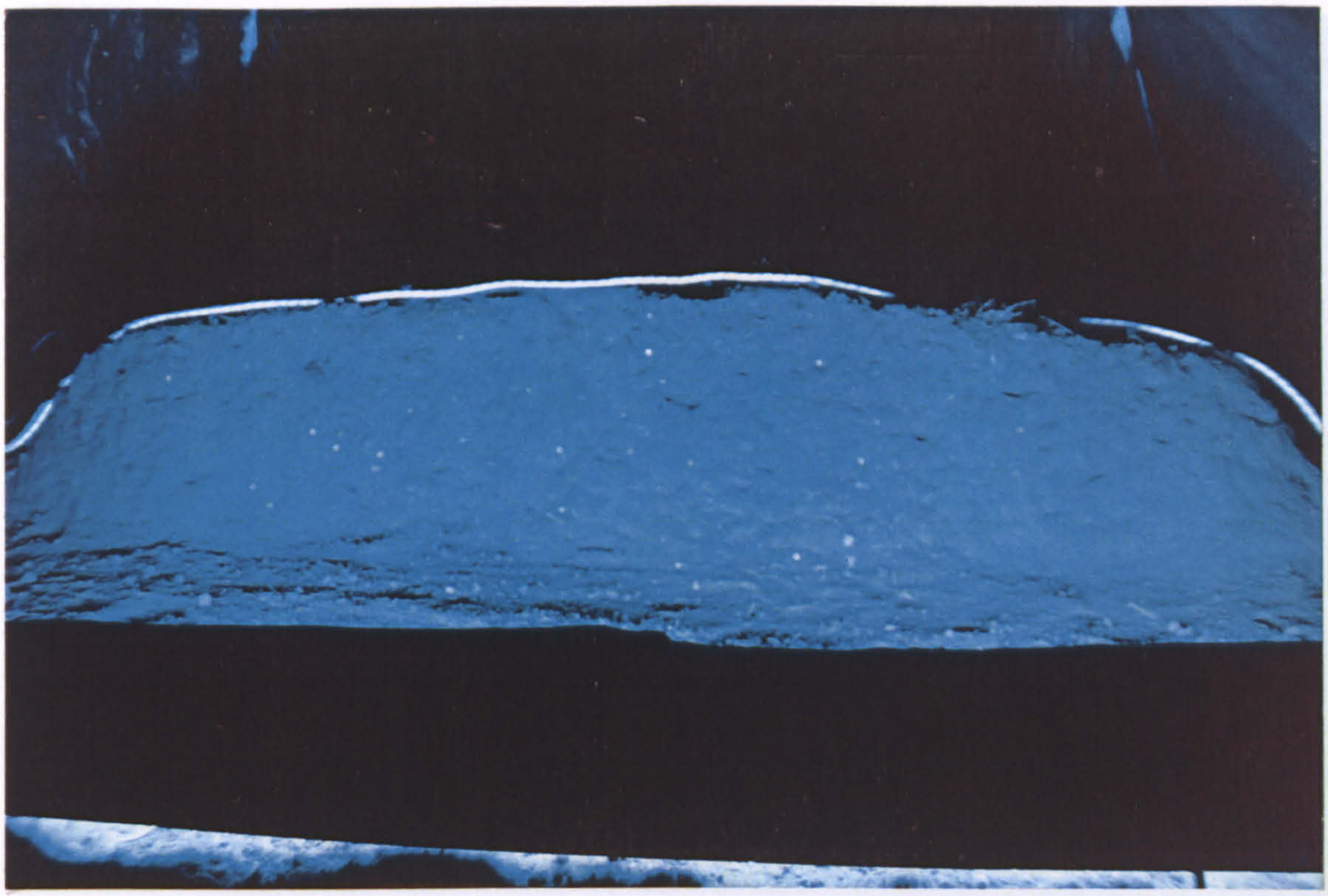


Plate 16. Soil profile photograph taken without filters.

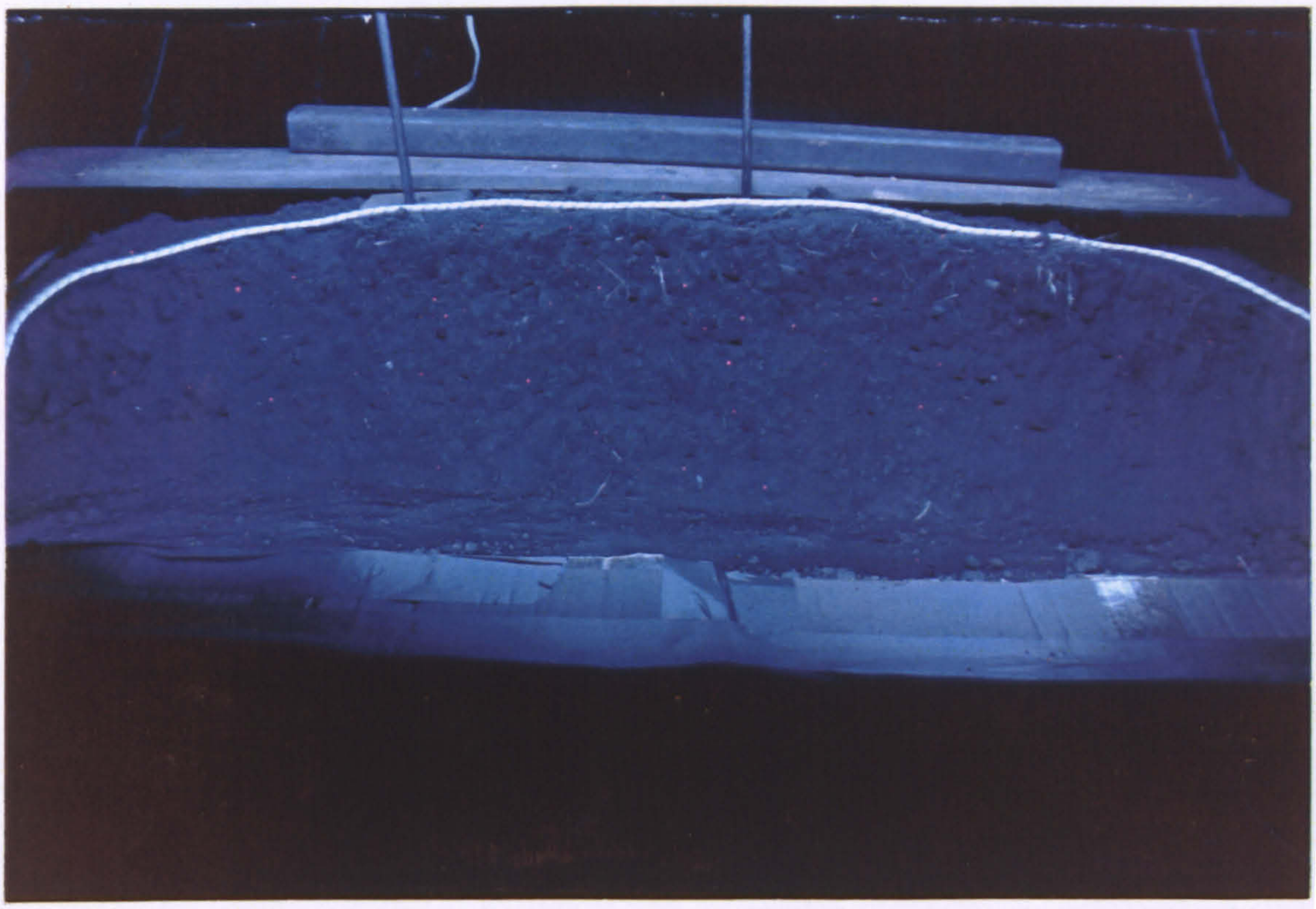


Plate 17. Soil profile photograph using a polarising filter.

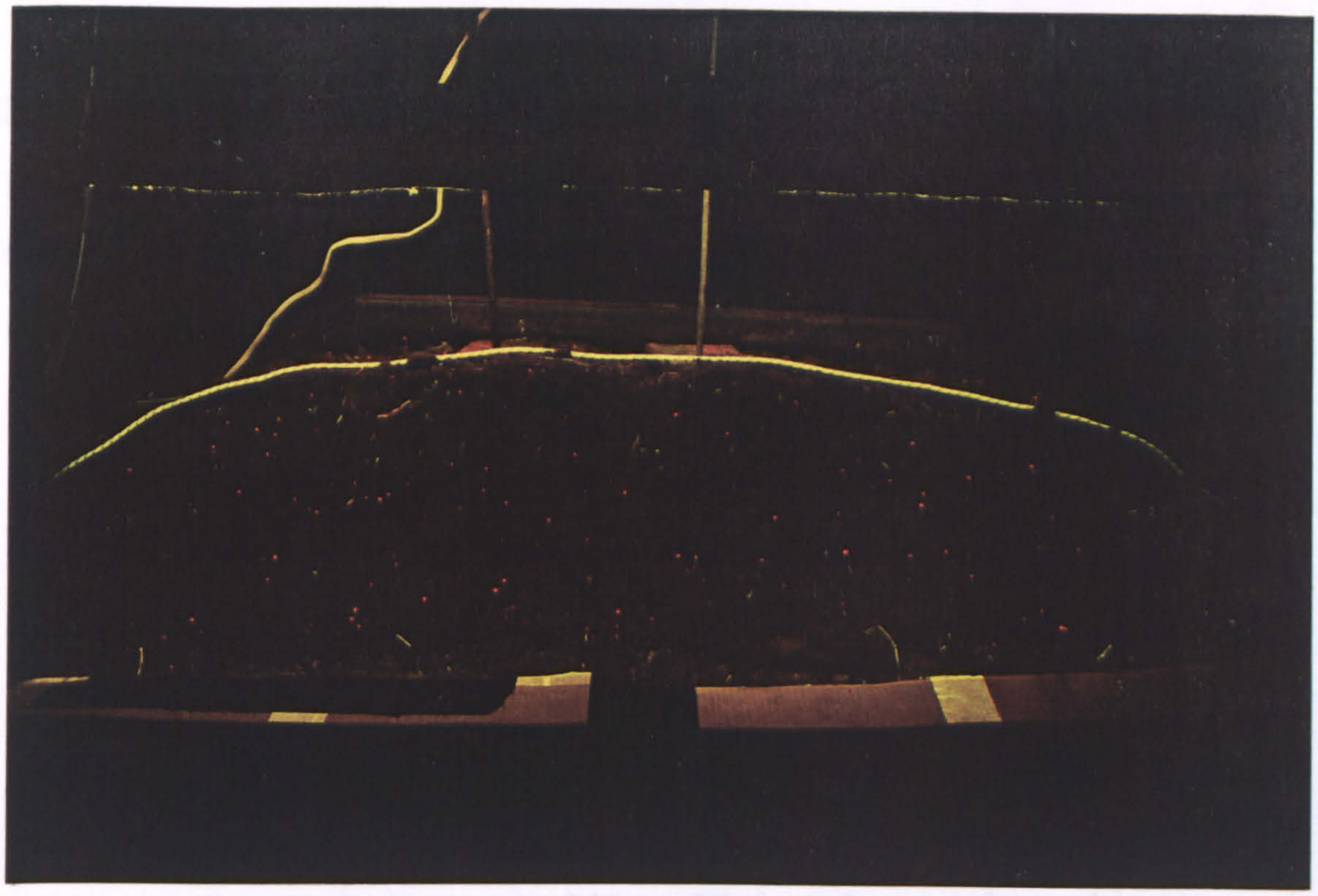


Plate 18. Soil profile photograph using an orange filter.

3.3.2 Tracer granule incorporation results

Plate 19 shows a soil profile after tracer granules were incorporated using the bed former followed by a second incorporation using the stone and clod separator. The tracer granules appear to have been incorporated evenly down to a depth of 35cm. This is probably due to the second incorporation by the stone and clod separator which appears to mix thoroughly soil from the top 15cm with that from the lower 15cm.

Plate 20 shows the distribution of tracer granules after incorporation by the stone and clod separator when the tracer was applied at the share / first web interface. It appears that tracer is incorporated into the top 15cm of the bed with no banding of the granules or dilution of the granules by incorporation to depths in excess of 30cm. Distinct clusters of granules can be seen which correspond to the numbers of outlets on the microband applicator.

Plate 21 shows the distribution of tracer in the soil profile after incorporation by a spiked rotavator. Tracer is incorporated evenly to a depth of 20cm with a small amount of tracer to a depth of 30cm. This may have been due to granule movement during soil profile excavation, but great care was taken to avoid this and it is likely that the machine was responsible. The Dowdswell Powavator used for this experiment was brand new and the unworn tines, when set to their maximum working depth would probably mix some granules to below 20cm. After incorporation by the spiked rotavator it was also noted that some granules were left on the surface of the bed.

Plate 22 shows the distribution of tracer granules in the soil profile after incorporation by the stone and clod separator with tracer applied half way up the first web. The incorporation of tracer appears shallow, with the majority of the tracer left on the bed surface and some incorporation to a depth of 5cm. This shallow incorporation of tracer probably occurred

because most soil fell through the first web of the stone and clod separator before it had travelled halfway up the web.

Plate 23 shows the distribution of tracer after the initial application by the vertical band applicator. The vertical bands of tracer 15cm deep can be seen. The soil was particularly dry where this treatment was performed which caused problems with tracer granule movement during profile excavation. Therefore the granules which appear to be deeply incorporated have most probably rolled down the profile.

Plate 24 shows the distribution of tracer after incorporation of the vertical bands by a roterra. The action of the roterra is horizontal, thereby mixing the vertical bands horizontally in the profile. This would seem to have been achieved with tracer uniformly incorporated to a depth of 15cm across the bed. The VBA blows a small quantity of tracer onto the soil surface when in operation. This is necessary to warn the operator if any tine outlets have become blocked. After further incorporation by the Roterra the amount of tracer on the soil surface was considerably reduced thereby reducing the risk of contamination to wildlife, such as birds.

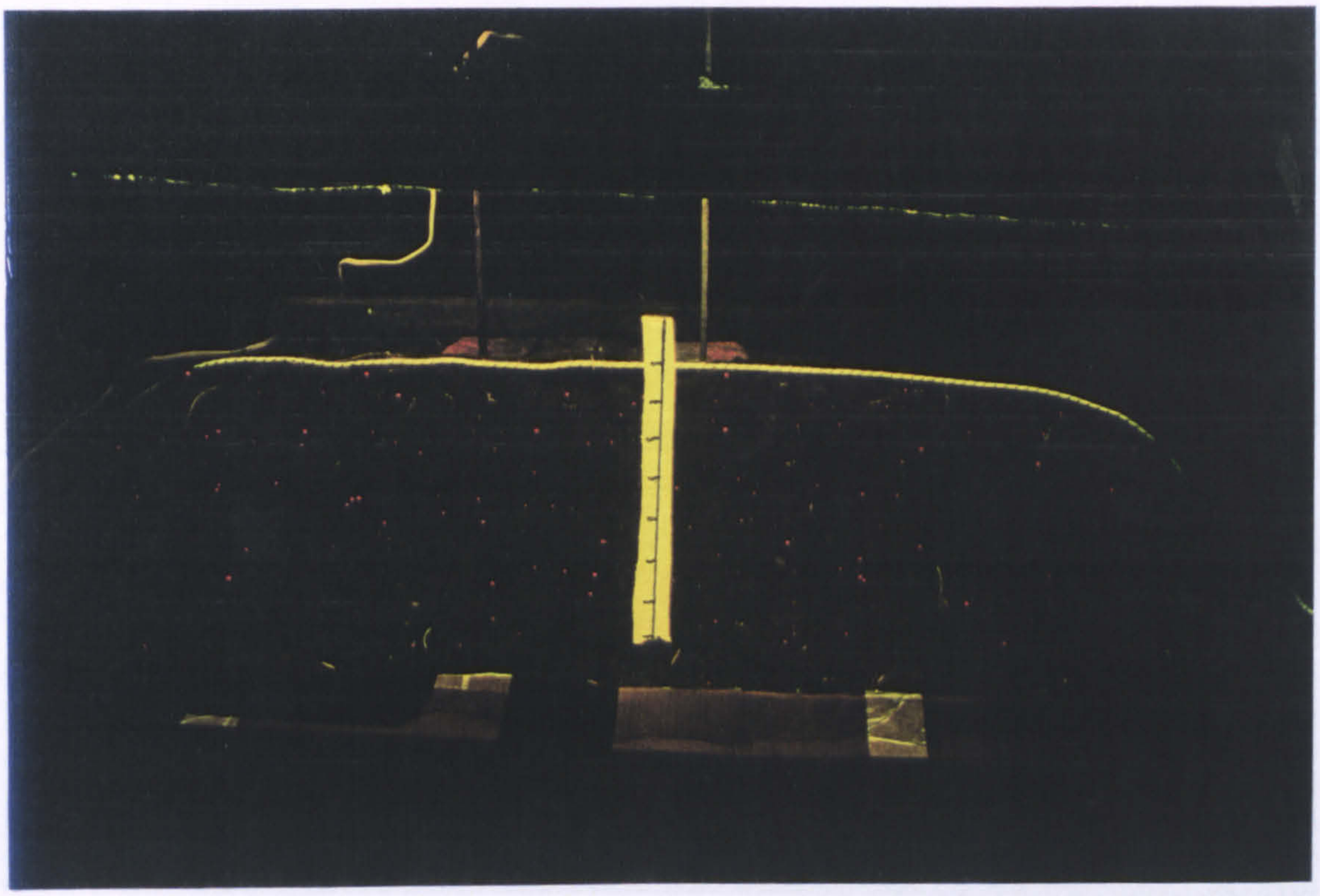


Plate 19. Incorporation by bed tiller followed by stone and clod separator.

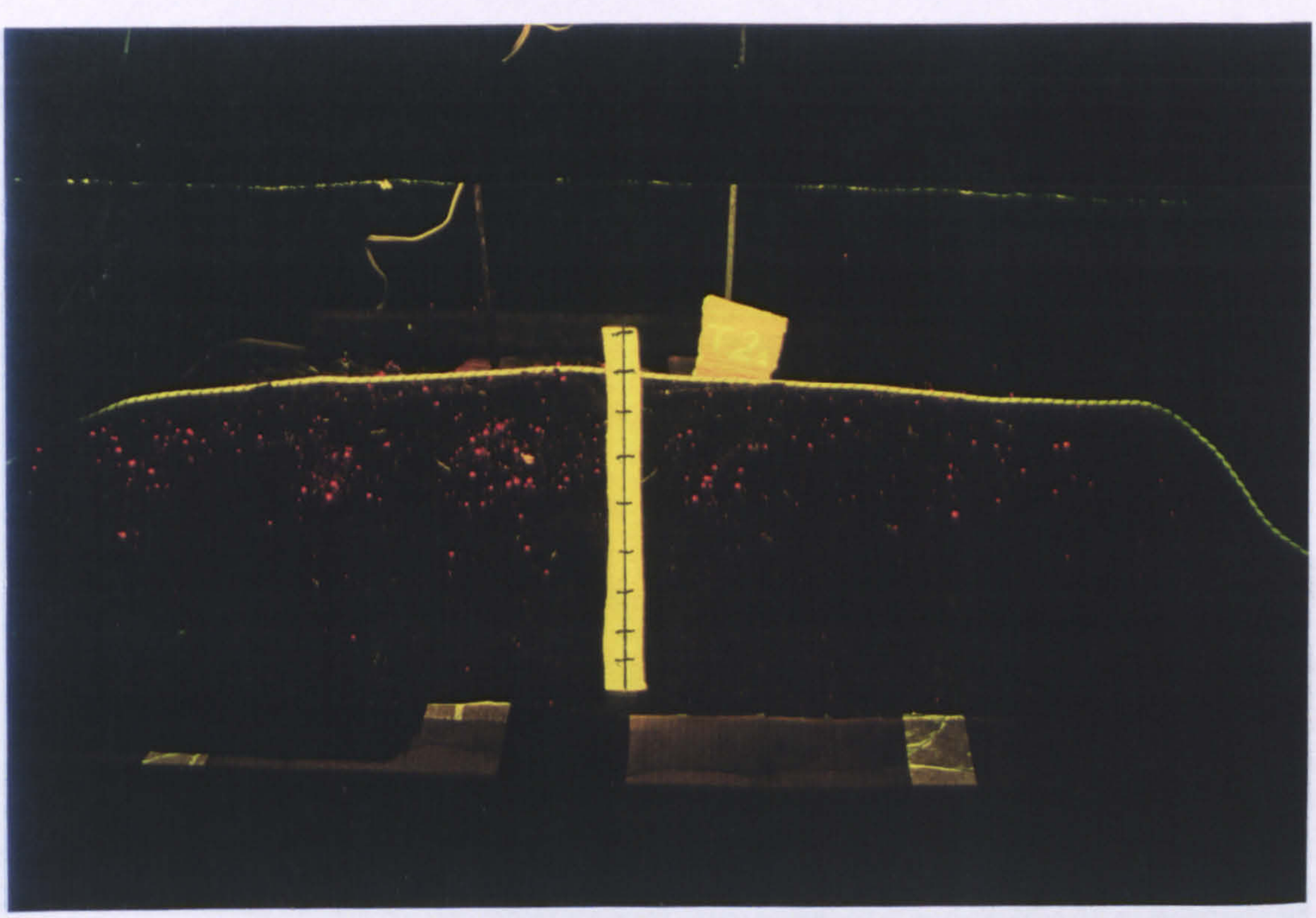


Plate 20. Incorporation of tracer by stone and clod separator when tracer applied at the share / first web interface.

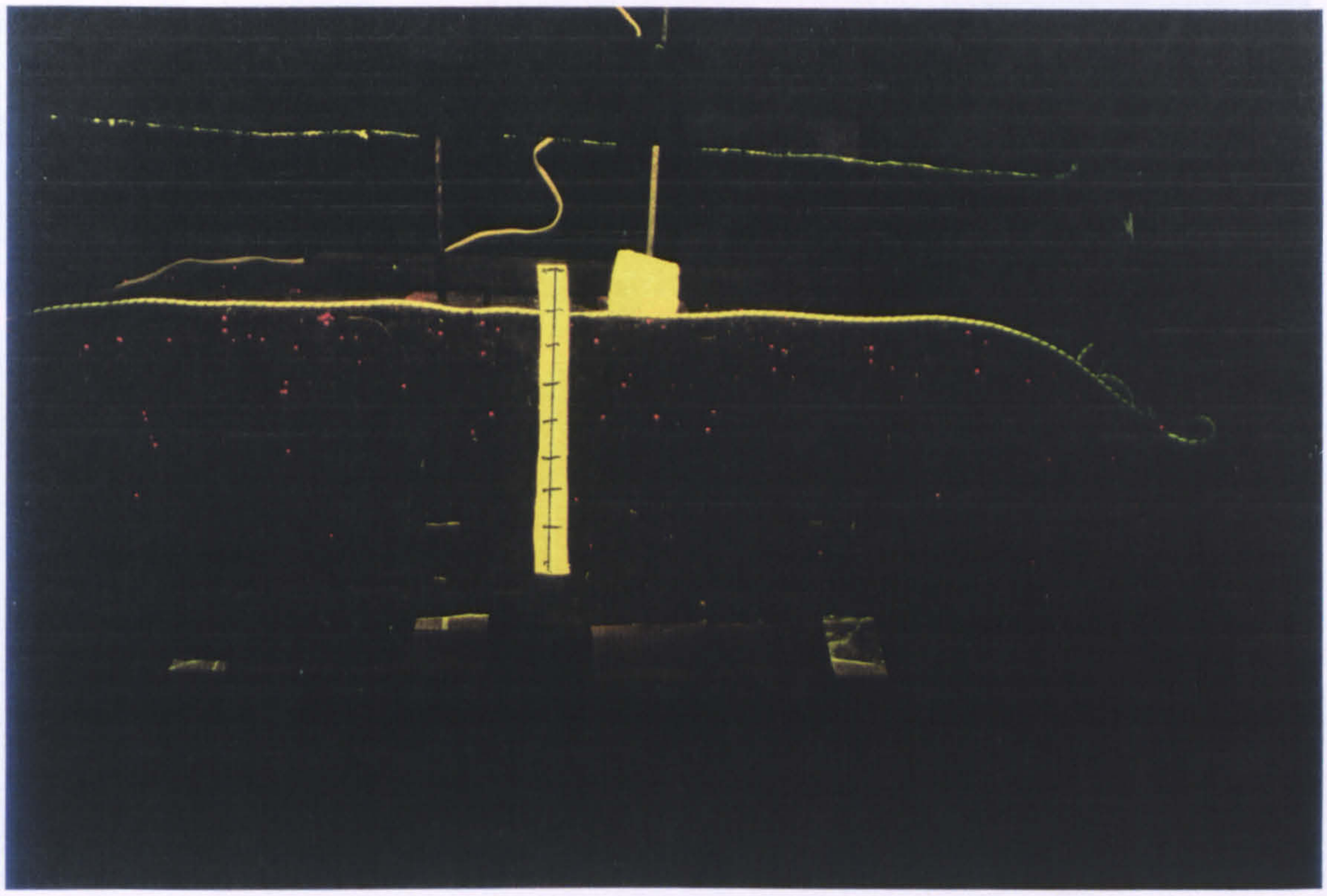


Plate 21. Incorporation of tracer by spiked rotavator.

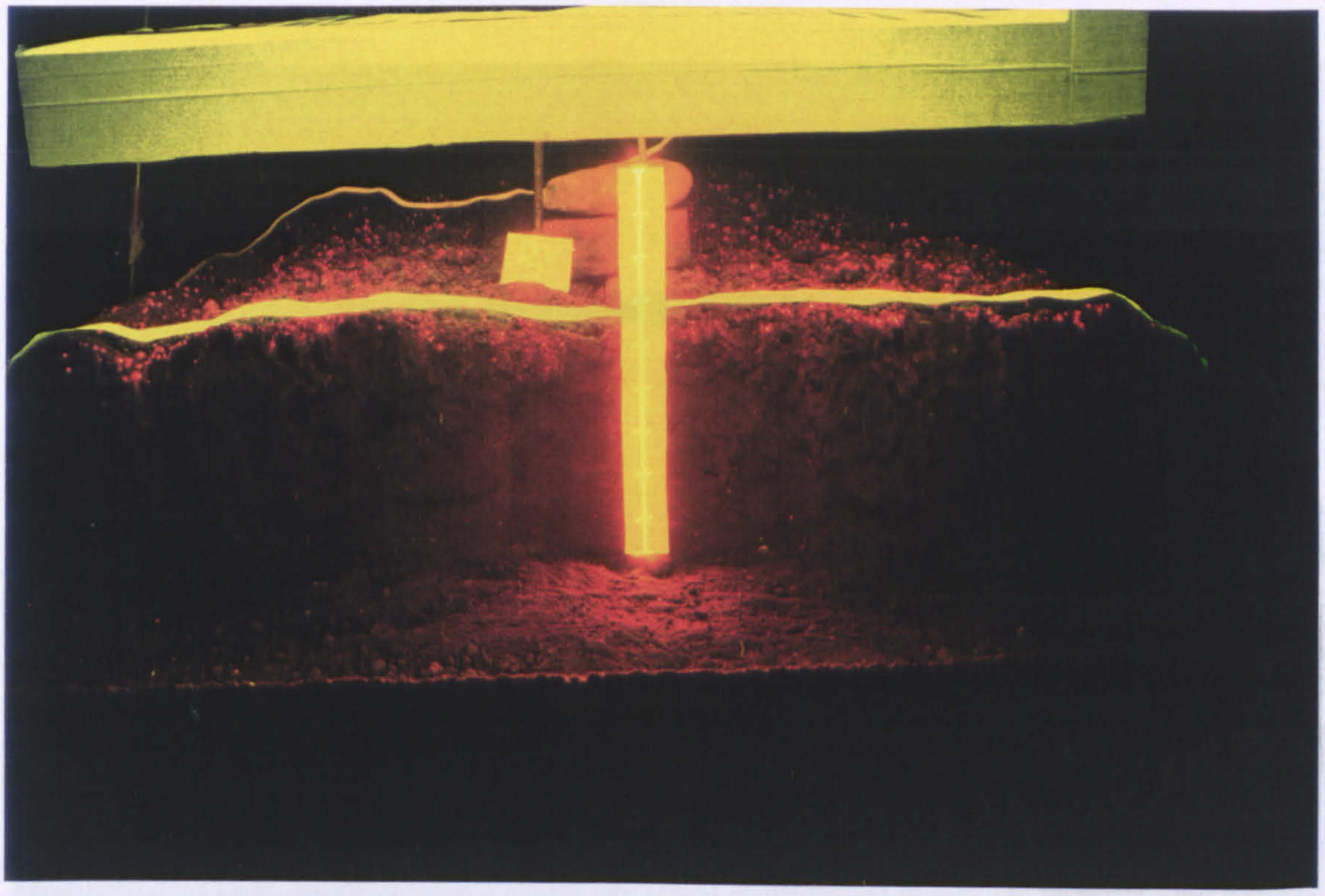


Plate 22. Incorporation of tracer by stone and clod separator with application of tracer half way up the first web.

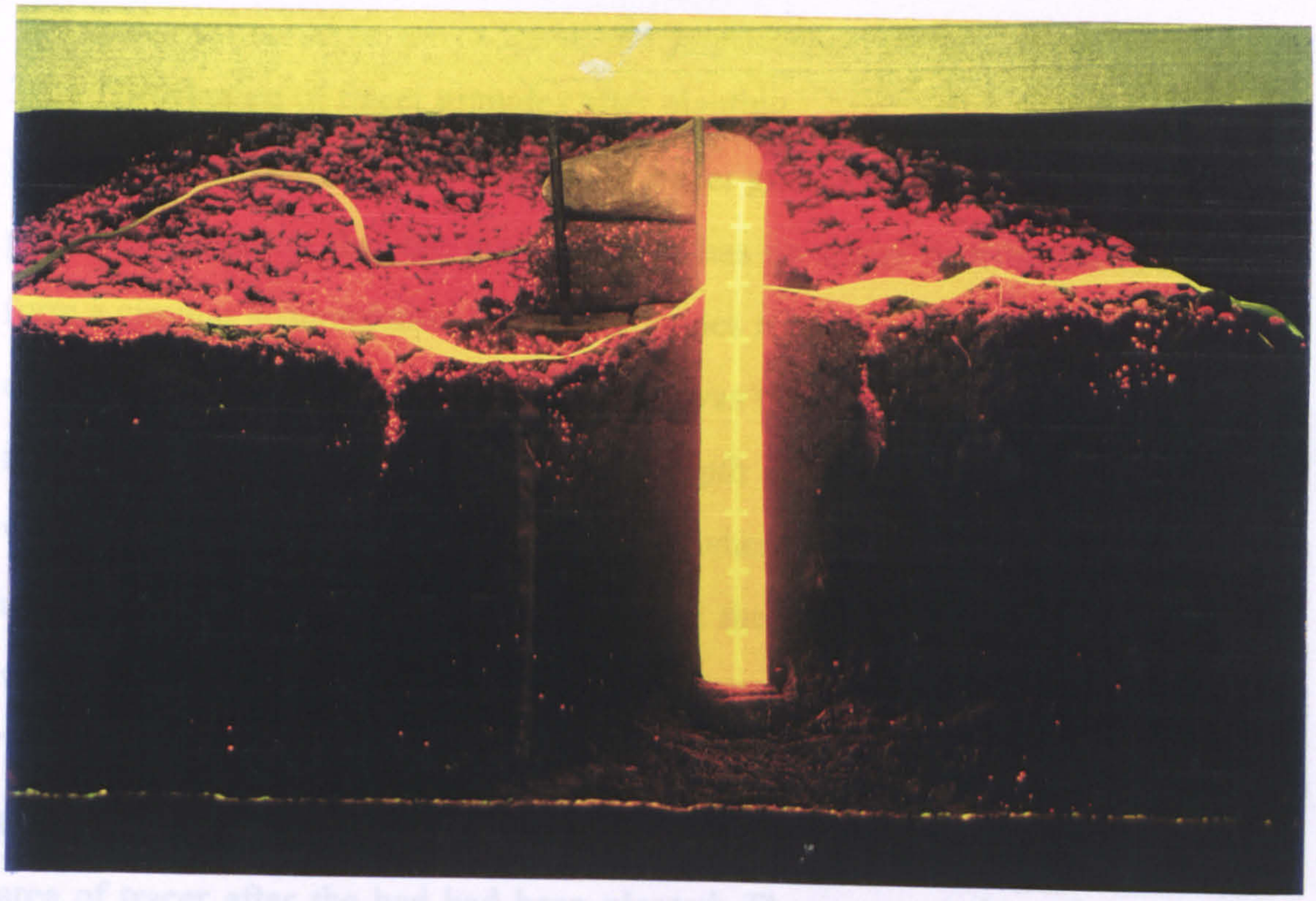


Plate 23. Incorporation of tracer by VBA.

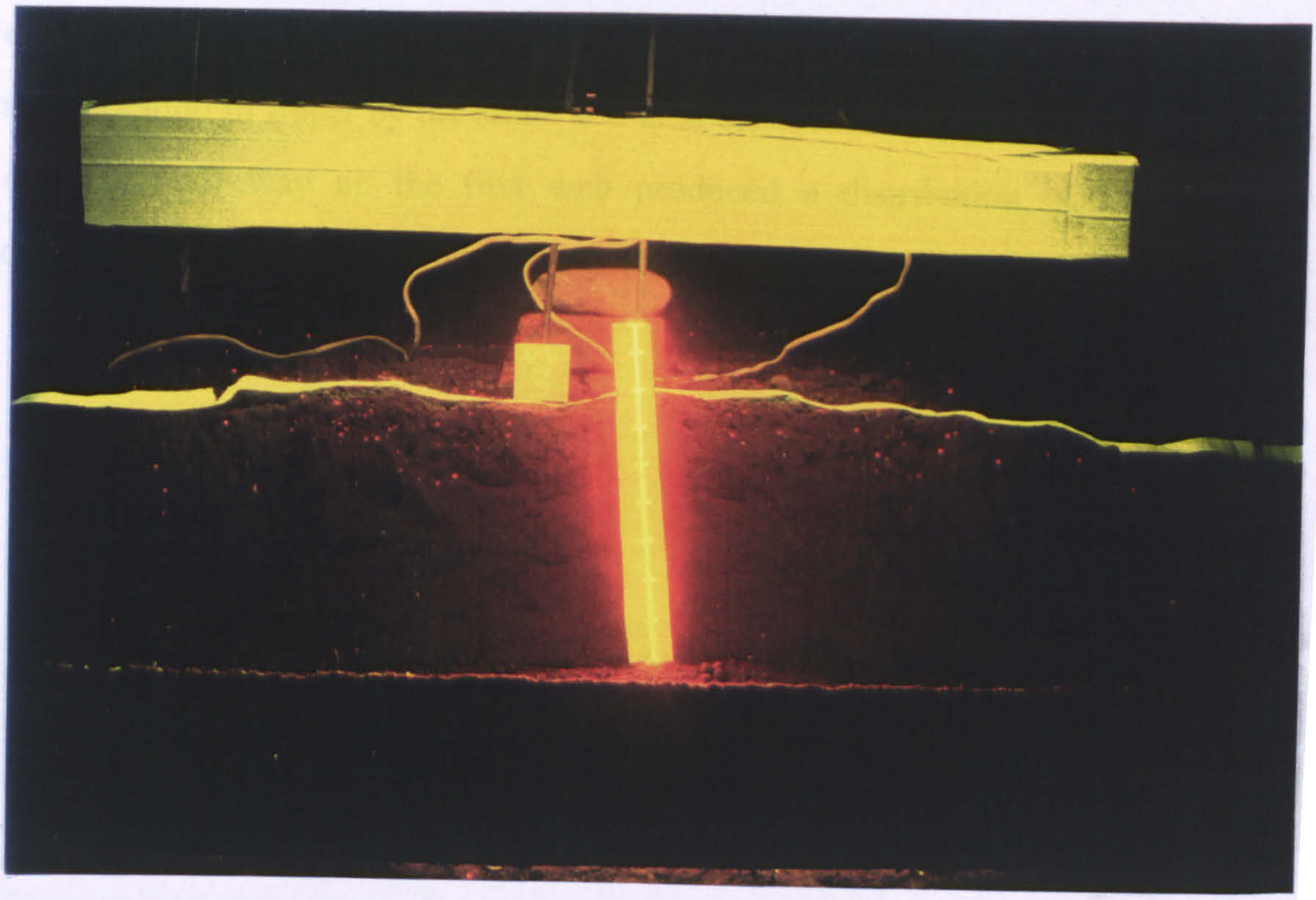


Plate 24. Incorporation of tracer by VBA then roterra.

3.3.3 Distribution of tracer granules after planting

Plates 25 to 29 show the distributions of tracer granules after the beds were planted. The action that the potato planter has on tracer distribution is to form a circular accumulation of tracer granules in the top of the ridge. This area is approximately 15-20cm deep. This was the case for treatments that incorporated the tracer uniformly to a depth of 15-20cm namely the vertical band applicator / Roterra (Plate 25 where the effect is particularly noticeable), spiked rotavator (Plate 26) and the stone and clod separator when application occurred at the front of the machine (Plate 27).

Treatments that did not produce the initial uniform distribution of tracer in the top 15-20cm of the soil profile did not subsequently produce a circular area of tracer after the bed had been planted. The incorporation of tracer initially by a bed former, followed by a second incorporation by a stone and clod separator produced a deep distribution of tracer and the distribution in the planted bed was also deep with tracer uniformly dispersed in the profile down to 40cm (Plate 28). The shallow incorporation of tracer by the stone and clod separator with the application of tracer half way up the first web produced a distribution of tracer confined to the top outer sides of the planted bed (Plate 29).

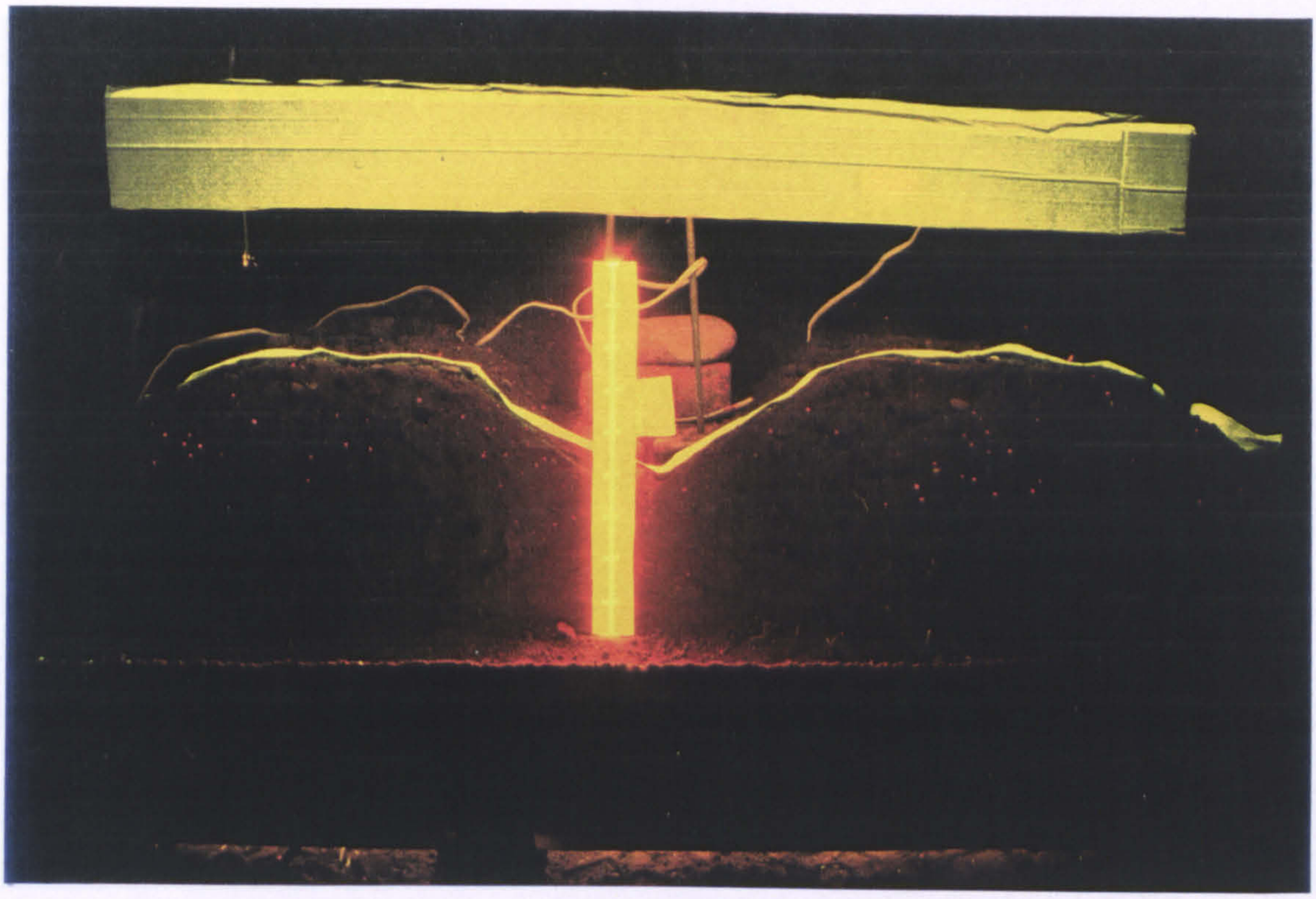


Plate 25. Distribution of tracer in planted bed after incorporation by the vertical band applicator / roterra.

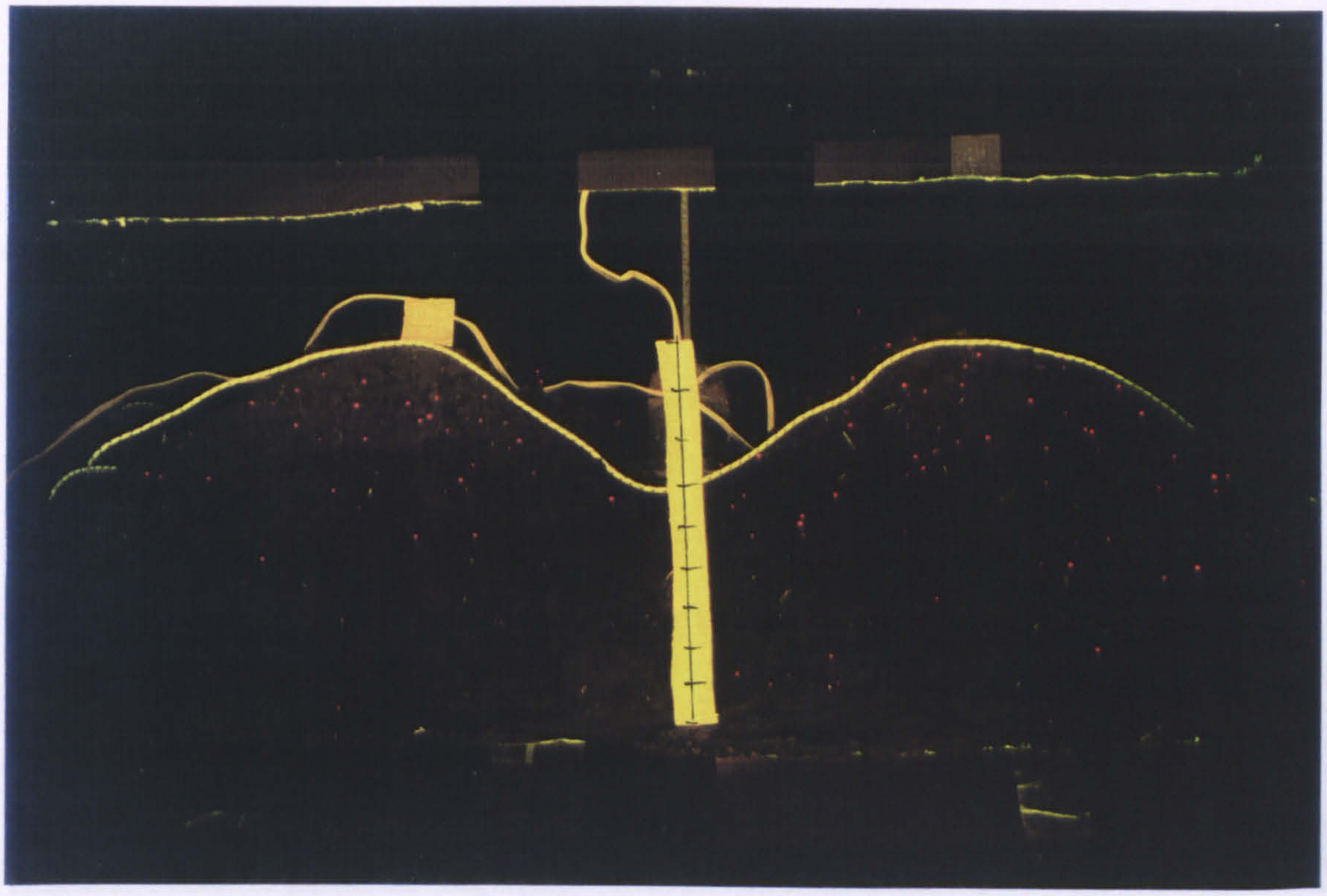


Plate 26. Distribution of tracer in the planted bed after incorporation by the spiked rotavator.

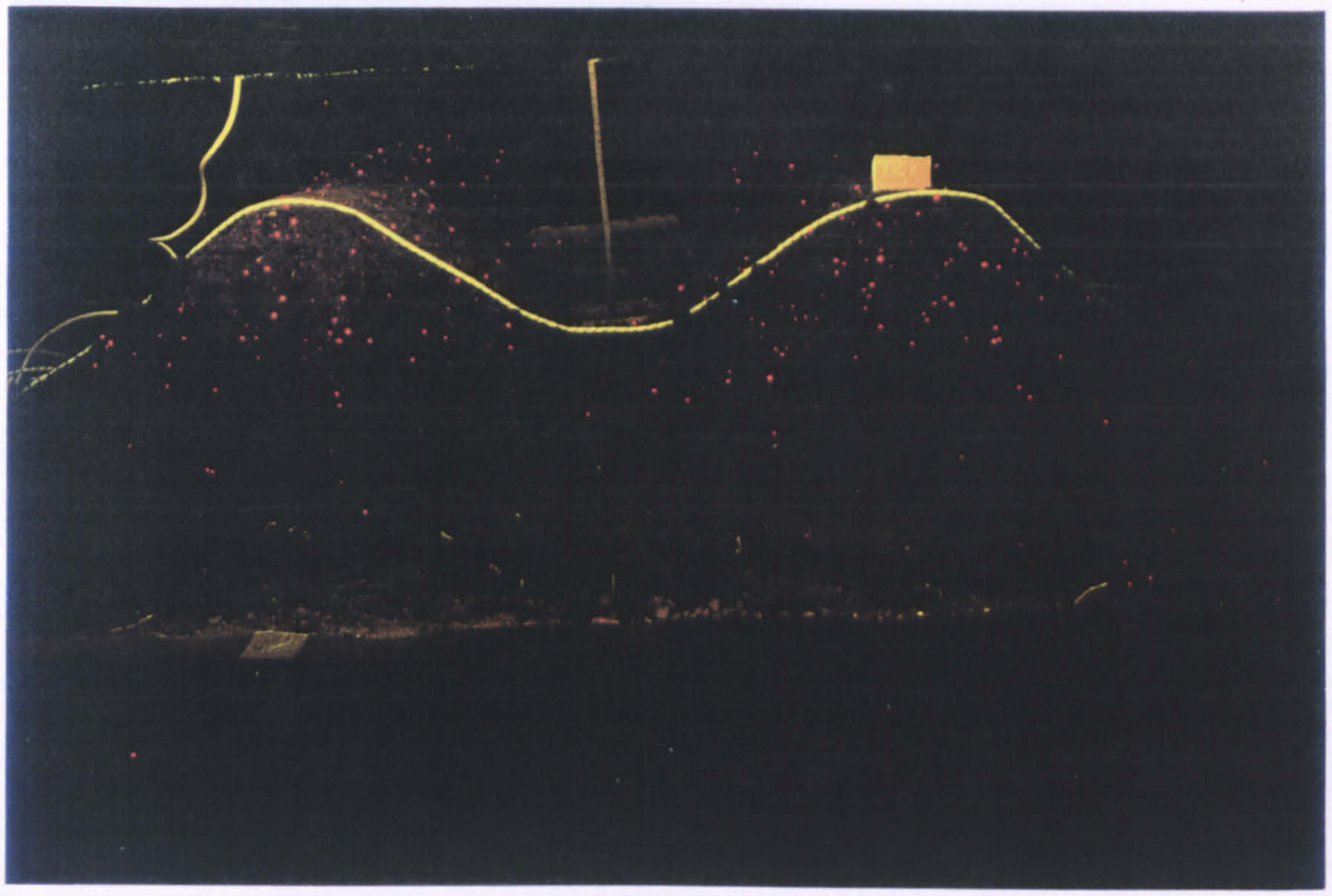


Plate 27. Distribution of tracer in the planted bed after incorporation by the stone and clod separator applying tracer at the share / first web interface.

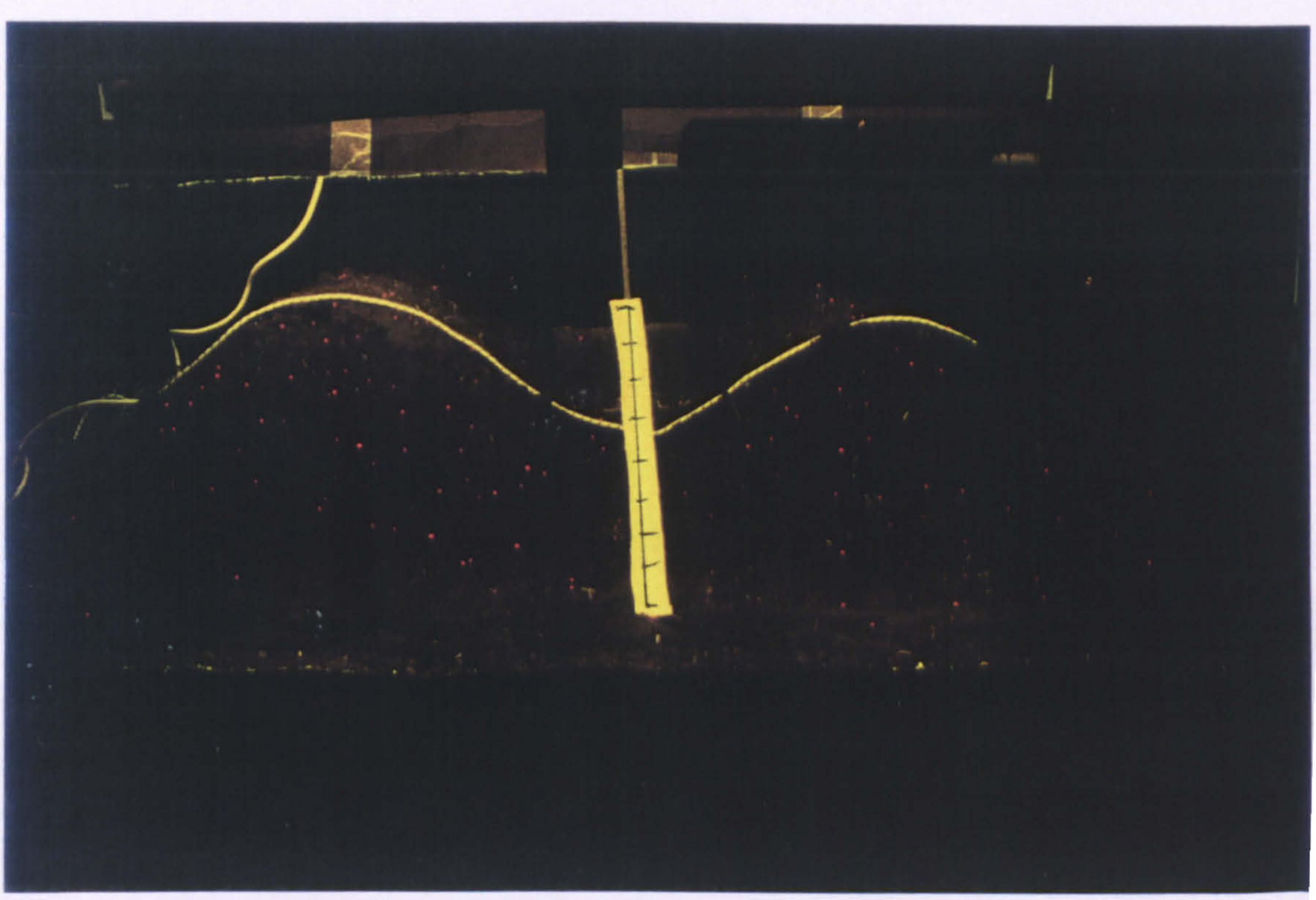


Plate 28. Distribution of tracer in the planted bed after an initial incorporation by the bed former followed by the stone and clod separator.

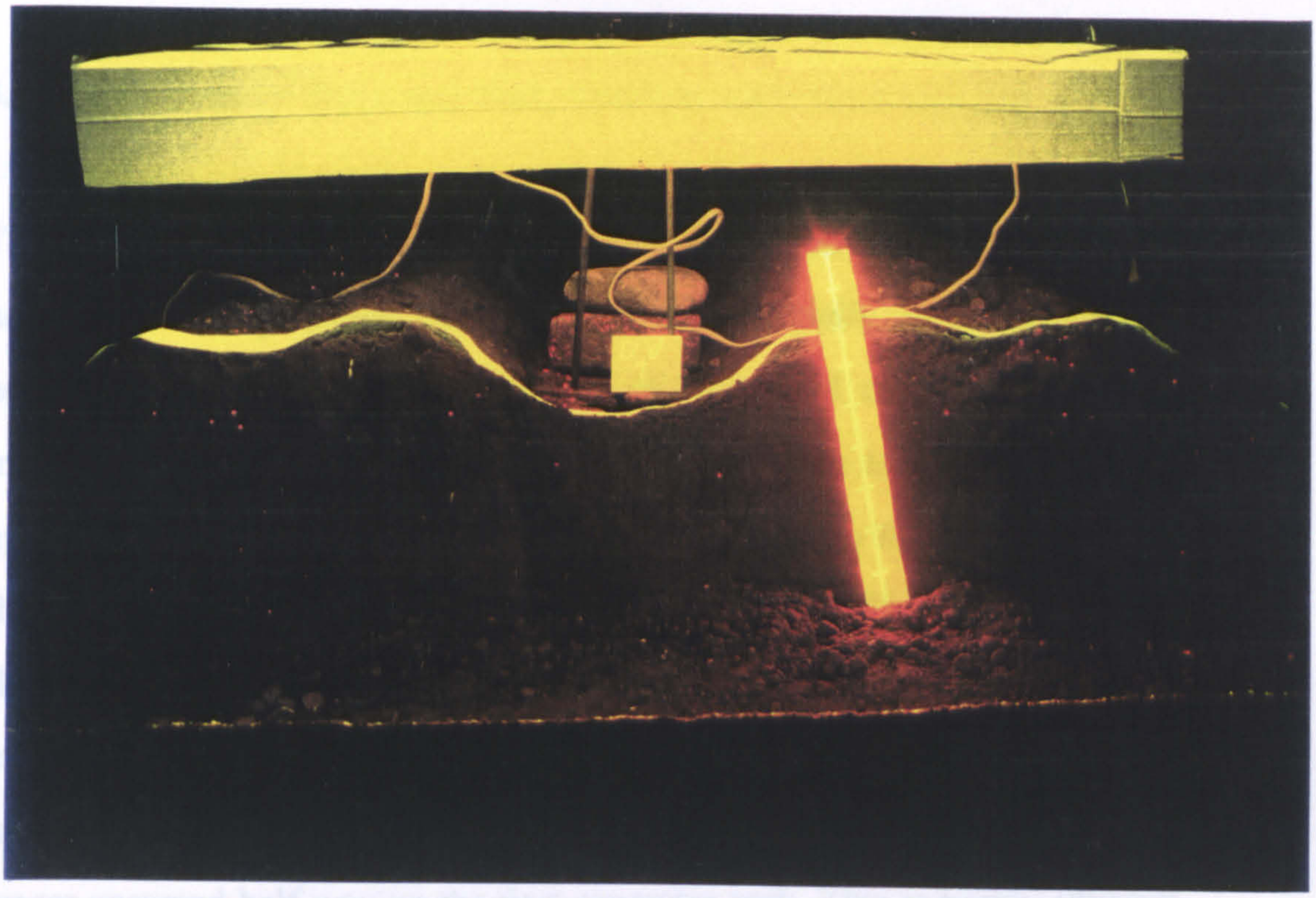


Plate 29. Distribution of tracer in the planted bed after incorporation by the stone and clod separator applying tracer half way up the first web.

3.4 Conclusions

The results shown provide clear evidence that differences exist between nematicide incorporation methods used commercially in the UK. The advice currently available on the use of stone and clod separators for incorporating granular nematicides may be correct in terms of where the application of a nematicide should occur in relation to the first web ie. at the junction of share and first web (Anon, 1995b). The action of over dilution or banding of nematicides by stone and clod separators in this experiment was a problem when the stone separator provided a second incorporation after that produced by a bed tiller, or if the application of tracer occurred half way up the first separator web. This is highly relevant to growers as applying and incorporating granular nematicides first by a bed tiller and then by a stone and clod separator has become popular, possibly as a result of uncertainty concerning the ability of stone and clod separators to incorporate granular nematicides correctly (Hancock, 1995). The stone and clod separator applying tracer halfway up the first web gave an uneven banded distribution of tracer in the planted bed, which may not provide either control of PCN or provide an economic improvement in yield. This method of incorporation has been highlighted as incorrect by the nematicide manufacturers and growers have now abandoned it. However, it was necessary to include this treatment in the experiment to ensure that the advice given to growers was correct. The double incorporation of tracer by the bed tiller followed by a stone and clod separator gave such a deep distribution of tracer that it is likely that an active ingredient would be diluted in too great a volume of soil. The consequences of possible dilution will be examined in later chapters dealing with field experiments in which all the methods used in this

chapter, except the stone and clod separator applying tracer half way up the first web, will be used.

4.0 CHAPTER 4

FIELD EXPERIMENTS 1 AND 2

4.1 Introduction and Preliminary Soil Sampling For Field Experiment 1, 1994

4.1.1 Introduction

The nematicide incorporation experiment (Field Experiment 1) was sited at the farm of Messrs Brian and Gordon Maddock, Great Bolas, Shropshire. The farm is 393 ha in size and has a rotation of potatoes, winter wheat, sugar beet, winter wheat and carrots. The farm has a problem with PCN, the populations of which are predominantly *Globodera pallida* (Mr B. Maddock *pers comm.*).

4.1.2 Methods

The field chosen for the Experiment has a history of high PCN population densities. A routine soil analysis conducted by the local DuPont crop protection advisor had shown counts of 50 eggs/g of soil before the field was last used to grow potatoes. This gave an indication that a moderate to high population density could now be expected. To map the distribution of PCN on a level area of uniform soil type within the field, twenty five squares of 20m x 20m covering an area of 1 ha (see Fig. 7) were marked out. Thirty cores (2.5cm x 22.5cm) were taken from equally spaced points in a systematic manner from each square. The cores were bagged, and then air dried at 25°C for 5 days. After drying, the soil was forced with a wooden block through a 4mm sieve to remove stones and coarse gravel. The soil was mixed thoroughly before sub-samples of 200g were taken. The sub-samples were then washed through a Fenwick can to extract any cysts using the method described by Shepherd (1986). The float recovered was then air dried and any cysts found were counted using the Fenwick counting tray (Fenwick, 1940). The first fifty round cysts to be counted were removed and soaked in 1ml of distilled water overnight. The soaked

cysts were then crushed to release any eggs and juveniles (Reid, 1955), which were then counted in aliquot samples using a version of Fenwick's counting slide. A suspension of the crushed cysts and their contents was made up to 50 ml with water in a marked tube. The suspension was then agitated to dislodge eggs from cyst wall fragments and homogenise the suspension before a 5ml pipette was used to draw off, midway between the top and bottom of the liquid, sufficient suspension to fill one chamber of the counting slide. The eggs and juveniles were counted using a stereo microscope (x50 magnification) and a hand tally to record the unhatched eggs and free juveniles. PCN species present was identified as *G. pallida* using isoelectric focusing (Flemming and Marks, 1983).

4.1.3 Results

Results of the initial sampling as eggs/g of soil are shown in Fig. 7.

4.1.4 Comments

The egg counts from the initial survey are fairly uniform with an average population density of 52 eggs/g of soil. This moderate to high infestation level was considered suitable for the Experiment site. The size of Field Experiment 1 meant that the entire area was used.

A1 59	A2 85	A3 60	A4 36	A5 ◀ — Plot I.D. 55 ◀ — Pi eggs/g of soil
A6 57	A7 37	A8 32	A9 42	A10 35
A11 72	A12 57	A13 50	A14 64	A15 69
A16 52	A17 54	A18 43	A19 52	A20 51
A21 55	A22 28	A23 58	A24 45	A25 57

20m

20m

Fig.7 Diagram of initial sampling grid and associated population densities of *G. pallida* for Field Experiment 1.

Field Experiment 1: A comparison of techniques used for applying and incorporating granular nematicides for the control of the potato cyst nematode *Globodera pallida* on a sandy soil

4.2.1 Materials and methods

4.2.1.1 Experimental Design

The experiment was a randomised block, split plot design. The plot size was 10m long by 10.8m wide (6 rows). Each treatment was replicated five times (see Fig. 8).

4.2.1.2 Experimental Method

The experiment was carried out at Maddock's Farm, Shropshire. Details of the site are given in Table 2. Four methods of incorporating the nematicides were compared:

1) Spiked rotavator incorporating nematicide.

2) Bed tiller applying and incorporating nematicide followed by destoner which gave a further incorporation.

3) Destoner applying and incorporating nematicide.

4) Vertical band application followed by Roterra.

These methods, as described in Chapter 3, provided similar incorporation of tracer granules in the planted bed, with the exception of the destoner giving a second incorporation of tracer after the bed tiller, where the incorporation was deeper. Aldicarb and oxamyl were compared for each incorporation method. The two nematicides were applied as Vydate 10G (oxamyl 10% ww a.i. granules) and Temik 10G (aldicarb 10% ww a.i. granules) according to the manufacturers' recommendations. A control with no nematicide treatment was included for each incorporation method.

Table 2. Details of Field Experiment 1 site.

Location	Topsoil type	Depth of topsoil (cm) and subsoil type	Subplot area including discard rows (m^2)	Number of replicates
Great Bolas	Sandy Loam	30-60 Sandstone	108	5

4.2.1.3 Application and incorporation of nematicides

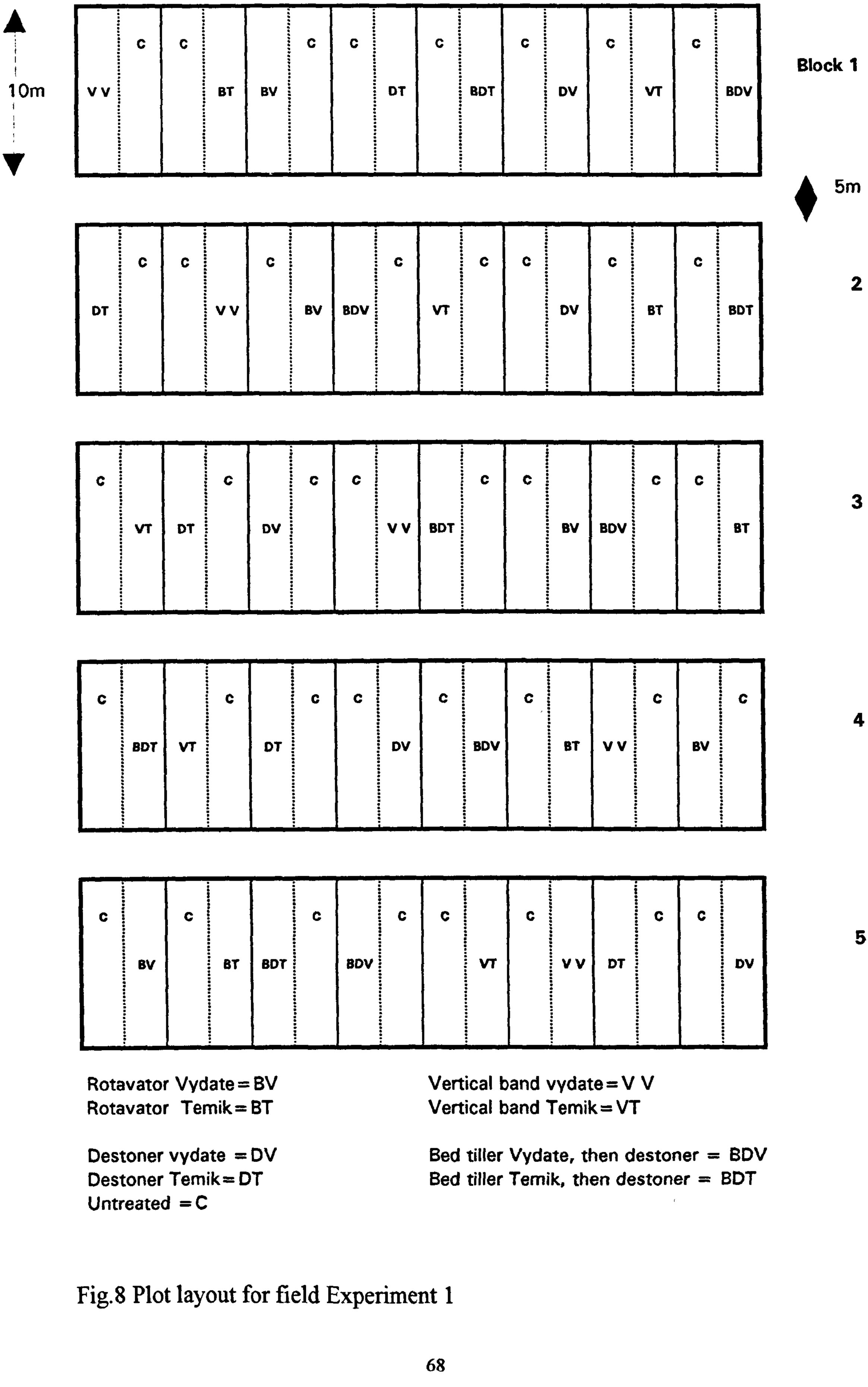
The nematicides were applied using a Horstine Farmery Microband applicator and the Vertical band applicator as described in Chapter 2. Nematicides were then incorporated using a Grimme Colt destoner, a Roterra after the vertical band applicator , a spiked rotavator, or a bed tiller followed by the destoner (see Chapter 2).

4.2.1.4 Planting

Plots were mechanically planted with the cultivar Santé on the 29/4/94. Super elite seed tubers were planted 30cm apart in 90cm rows. Crop husbandry after planting followed that of a commercial crop. Details of crop inputs can be found in Appendix 2.

4.2.1.5 Percentage ground cover

The percentage ground cover from each plot was measured fortnightly from the 3/6/94 to the 18/7/94. A viewing frame as described by Evans , Parkinson and Trudgill (1975) was used to take the measurements.



Rotavator Vydate = BV
Rotavator Temik = BT

Destoner vydate = DV
Destoner Temik = DT
Untreated = C

Vertical band vydate = V V
Vertical band Temik = VT

Bed tiller Vydate, then destoner = BDV
Bed tiller Temik, then destoner = BDT

Fig.8 Plot layout for field Experiment 1

4.2.1.6 Growth analysis

Six weeks after planting, one whole plant was removed from each plot, dissected and the following information obtained;

1. Fresh and dry weight of tubers with diameter of 10mm or over.
2. Fresh and dry weight of the stems and leaves.
3. Fresh and dry weight of the stolons.
4. Fresh and dry weight of the roots.

A 2g subsample of the fresh root system was preserved in formal acetic alcohol (FAA; Hooper, 1986) to enable root invasion by PCN to be determined at 6 weeks.

4.2.1.7 Harvesting

The haulm from the plants in the two centre rows was removed by hand on the 12/9/94. A 5m length of the harvest rows from each plot was then lifted by hand using a fork between 13/9/94 and 18/9/94. Tubers were mechanically riddled over 45mm and 65mm webs. Tubers greater than 45mm constituted ware yield.

4.2.1.8 Soil sampling for potato cyst nematode

Bulk samples of 60 soil cores (1.0cm diameter and 9cm deep) were taken in a zig zag fashion from the middle two rows of each subplot, and also from the furrows adjacent to the middle two treated rows. Row samples were taken immediately after planting and after harvest, furrow samples were taken immediately after planting and immediately before harvest. The number of eggs in soil samples was estimated by standard methods. This core size was used to give more sample points per plot than that used in the preliminary sampling.

4.2.1.9 Data analysis

Data from the field Experiment were analysed using analysis of variance on Genstat V mark 4.03

4.2.2 Results and discussion

4.2.2.1 Abbreviations

BV=Bed tiller incorporating Vydate
BT=Bed tiller incorporating Temik
BDV=Bed tiller incorporating Vydate followed by incorporation by destoner
BDT=Bed tiller incorporating Temik followed by incorporation by destoner
DV=Destoner incorporating Vydate
DT=Destoner incorporating Temik
VV=Vertical band applicator followed by roterra incorporating Vydate
VT=Vertical band applicator followed by roterra incorporating Temik

4.2.2.2 Plant Emergence

Table 3. Mean percentage emergence for Field Experiment 1 (7/6/94)

TREATMENT	% EMERGENCE	
	+NEMATICIDE	-NEMATICIDE
BV	65.0	67.8
BT	65.0	69.2
BDV	69.2	67.2
BDT	67.4	66.4
DV	66.6	67.6
DT	68.8	71.0
VV	67.8	64.6
VT	66.0	66.8
MEAN	66.9	67.6

S.E.=2.76 (7 D.F.) C.V%.=8.2

Table 3 shows the mean percentage emergence counts for Field Experiment 1 taken on the 7th June 1994. The variability of the emergence is low and no significant differences occurred between treatments, indicating that the seed used was good quality planted at a uniform depth and uniform in size. Plant populations in each plot were uniform, thereby reducing error in interpretation of later observations.

4.2.2.3 Six week growth analysis

Table 4. Results from destructive plant sampling six weeks post planting (10/6/94)

Incorp method	nematicide	Dry weight g					Juveniles per g of root
		Haulm	Root	Stolon	Tuber	Total	
BV	+	34.03	1.35	0.71	28.57	64.7	463
BV	-	16.69	0.78	0.25	12.16	30.0	1034
BT	+	35.91	1.30	0.70	23.93	61.8	462
BT	-	21.94	1.09	0.30	14.41	37.7	1333
BDV	+	24.21	0.92	0.54	18.37	44.0	730
BDV	-	18.62	0.91	0.50	10.90	30.9	1127
BDT	+	31.34	1.10	0.66	17.53	50.6	309
BDT	-	23.68	1.06	0.48	15.62	40.8	1123
DV	+	32.45	1.23	0.91	19.24	53.8	638
DV	-	19.69	0.73	0.42	15.68	36.5	826
DT	+	35.13	1.22	0.68	20.99	58.0	394
DT	-	19.75	0.94	0.30	15.47	36.5	1001
VV	+	30.90	0.95	0.72	18.55	51.1	464
VV	-	17.85	0.89	0.37	1 3.01	32.1	1170
VT	+	29.09	0.96	0.66	20.31	51.0	678
VT	-	24.28	0.95	0.51	21.99	47.7	1008
S.E	(7 D.F)	3.437	0.187	0.149	3.188	6.15	145.7
C.V%		29.6	41.3	56.6	39.8	30.1	38.9

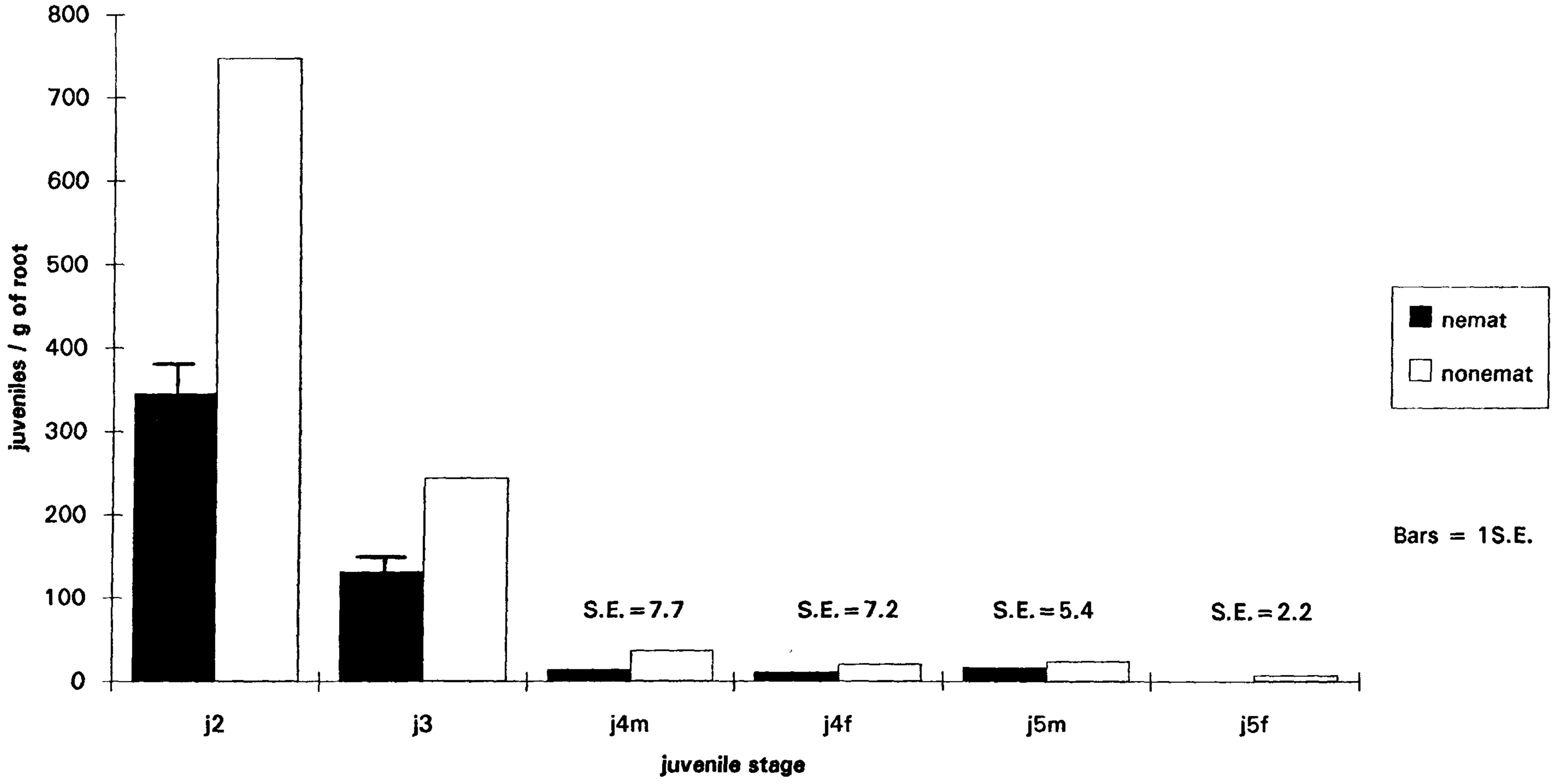
Table 5. Nematicide use and six week destructive plant sampling

	Plant dry weight (g)					Juveniles
nematicide	haulm	root	stolon	tuber	total	per g root
+	31.63	1.128	0.699	20.94	54.4	517
-	20.31	0.918	0.391	14.90	36.5	1078
S.E(1D.F.)	1.215	0.0667	0.0487	1.129	2.17	54.9
C.V%.	20.9	28.6	46.0	28.1	21.5	28.6

Six weeks after planting, no significant differences in plant dry weight or numbers of juveniles per g of root occurred between incorporation methods or type of nematicide (Table 4). Therefore, means for treated and untreated plots are presented in Table 5, where it can be seen that the numbers of nematodes invading the roots in nematicide treated plots is significantly lower ($P \leq 0.05$) with approximately half as many nematodes as in untreated plots. This lower root invasion for treated plots is reflected in the plant dry weights with treated plots having significantly higher ($P \leq 0.05$) dry weights than untreated plots. However, there was no significant differences in root dry weights between treated and untreated plots. This may in part be due to the difficulty of collecting all of the roots of a single potato plant growing in the field.

Figure 9 shows the mean numbers of nematodes per g of root that have invaded the potato roots in nematicide treated and untreated plots. The partial resistance of the cultivar Santé is highlighted in this figure with no fifth stage female nematodes present in the roots from nematicide treated plots, and few from untreated plots compared with the occurrence of fifth stage male nematodes.

Figure 9. Numbers of each nematode developmental stage /g of root six weeks after planting for nematicide treated and untreated plots



4.2.2.4 Yield

Mean total yield, ware yield and percentage ware yield are given in Table 6. Individual tuber yields of <45mm, 45-65mm and 65+mm are given in Table 8.

The results for total tuber yield (Table 6) showed no significant differences between treatments. However, overall nematicide treated plots had a significantly higher mean yield ($P \leq 0.05$) of 48.8t/ha than untreated plots, which gave a total mean yield of 39.3t/ha (Table 7). Mean ware yields (Table 6.) showed no significant differences between individual treatments, but the nematicide treated plots collectively gave significantly higher mean ware yields ($P \leq 0.05$) of 44.7t/ha compared with 36.1t/ha from untreated plots (Table 7). Mean percentage ware showed no significant differences between individual treatments or between nematicide treated and untreated plots.

Yield data split into respective grade components showed differences in the <45mm grade and the 65+mm grade (Table 8). The broadcast and rotavation of Vydate (BV), and the vertical band applicator applying Vydate (VV) gave mean <45mm yields of 5.0t/ha and 5.1t/ha respectively. This was significantly higher ($P \leq 0.05$) than the vertical band applicator (VT) applying Temik, which gave a mean <45mm yield of 3.4t/ha. There were no significant differences between the other treatments. These results suggest that Vydate incorporated with a rotavator or a vertical band applicator increases the yield of small potatoes compared to Temik incorporated using the vertical band applicator. Overall the use of nematicide significantly ($P \leq 0.05$) increased the yield of < 45mm tubers (Table 9).

The 45-65mm component of tuber yield showed no significant differences between treatments, but overall the use of a nematicide increased the yield of this grade significantly ($p<=0.01$), (Table 9).

Table 6. Mean yields for Field Experiment 1.

Incorporation method	nematicide	total yield t/ha	ware yield t/ha	percentage ware
BV	+	45.8	40.8	89.1
BV	-	38.3	35.4	92.2
BT	+	45.2	41.3	91.0
BT	-	40.5	37.4	91.1
BDV	+	47.4	43.8	91.9
BDV	-	40.4	37.1	91.7
BDT	+	54.5	50.6	92.7
BDT	-	39.7	36.4	91.3
DV	+	49.8	45.5	91.2
DV	-	41.9	38.7	92.2
DT	+	49.7	45.8	91.9
DT	-	39.6	36.3	91.3
VV	+	48.5	43.4	88.4
VV	-	39.9	36.9	91.8
VT	+	49.3	45.9	92.8
VT	-	34.5	30.8	89.4
S.E.(7D.F)		3.14	3.52	1.41
C.V%		13.4	15.2	3.2

Table 7. Nematicide use and yield for Field Experiment 1

	nematicide		
	+	-	S.E (1 D.F)
Total yield t/ha	48.8	39.3	0.95
Ware yield t/ha	44.7	36.1	0.98
Percentage ware	91.1	91.4	0.47

Table 8. Tuber yield t/ha by grade for Field Experiment 1

Incorporation method	nematicide	<45mm	45-65mm	65+mm
BV	+	5.0	29.5	11.3
BV	-	2.9	24.8	10.6
BT	+	3.9	26.4	14.9
BT	-	3.1	24.7	12.7
BDV	+	3.5	28.3	15.6
BDV	-	3.2	25.7	11.4
BDT	+	3.9	28.9	21.7
BDT	-	3.2	26.8	9.7
DV	+	4.3	29.7	15.8
DV	-	3.2	27.0	11.7
DT	+	3.8	27.1	18.7
DT	-	3.3	25.9	10.4
VV	+	5.1	31.2	12.2
VV	-	3.1	26.0	10.9
VT	+	3.4	26.1	19.9
VT	-	3.8	24.1	6.7
S.E.(7D.F.)		0.33	1.74	2.71
C.V%.		19.3	13.5	31.0

Table 9. Nematicide use and tuber yield by grade for Field Experiment 1.

Tuber yield by grade	Nematicide +	Nematicide -	S.E.(1 D.F.)
<45mm t/ha	4.1	3.2	0.11
45-65mm t/ha	28.4	25.6	0.59
65+mm t/ha	16.3	10.5	0.67

The 65+mm component of yield showed differences between treatments (Table 8) incorporating Temik with a bedformer followed by a second incorporation by a stone and clod separator (BDT) gave a mean 65+mm

tuber yield of 21.7t/ha. The vertical band applicator incorporating Temik (VT) gave a mean yield of 19.9t/ha. These treatment yields of 65+mm tubers were higher than the vertical band applicator incorporating Vydate (VV) and the bed former incorporating vydate (BV) which gave the lowest 65+mm tuber yield of 12.2t/ha and 11.3t/ha respectively. However, there were no statistically significant differences between nematicide incorporation treatments.

Overall, the use of a nematicide increased the yield of the large 65+mm tubers for all treatments significantly ($P \leq 0.05$) with treated plots giving a mean 65+mm tuber yield of 16.3t/ha compared to 10.5t/ha for untreated plots.

These results for the 65+mm range suggest that using a nematicide improved the yield of the most lucrative tuber grade and that incorporation technique may further influence the yield of this grade.

4.2.2.5 Crop percentage ground cover

Differences in percentage ground cover between incorporation treatments and nematicide type did not occur over the sampling dates. Significant differences ($P \leq 0.05$) did occur between treated and untreated plots (Table 10), with treated plots consistently achieving greater ground cover than untreated plots. The greater ground cover on treated plots is likely to enable plants to intercept greater amounts of solar radiation which will ultimately result in increased yields given that other factors such as water and crop nutrients are not limiting. The increased ground cover in treated plots usually occurs because the root systems in these plots have been protected by nematicide treatment. This is likely to be the case here, but the root data from six week destructive sampling is inconclusive due to the difficulty of removing entire plant root systems from the field.

Table 10. Mean percentage ground covers and nematicide use

	Percentage ground cover			
Nematicide	3/6/94	17/6/94	3/7/94	18/7/94
+	6.6	38.8	77.3	91.5
-	4.0	28.8	63.3	77.3
S.E.(1D.F.)	0.39	0.85	1.11	1.14
C.V%.	30.1	10.8	9.2	6.4

4.2.2.6 Nematode population estimation

4.2.2.7 Within beds population changes

The mean Pi counts are shown in Table 11. The overall mean Pi value for Field Experiment 1 was 86 eggs/g of soil. No significant differences were found between blocks or plots. However, this is due to the high variability of the data. Uniformity of initial nematode population is important because it allows accurate comparisons to be made between treatments. However, PCN is generally not normally or uniformly distributed, tending to follow a Poisson or negative binomial distribution. Any treatment variation in normally distributed or uniformly PCN infested ground will have a low probability of being due to initial nematode population densities. As a result of high variability in these counts, log transformations of the counts will be presented in parentheses when analysing population data.

PCN populations after harvest of Field Experiment 1 showed no significant differences between incorporation treatments, nematicides (Table 11) or treated and untreated plots (Table 12). This is probably due to the partially resistant cultivar Santé reducing nematode multiplication regardless of nematicide treatment. However, the success of the partial resistance in

reducing nematode populations in this Experiment should be noted; the average initial PCN population density of 86 eggs/g of soil is likely to have increased dramatically if a tolerant cultivar had been grown. The mean final PCN population density of 69 eggs/g of soil shows a slight reduction in the population but, most importantly, the population has not increased, so leaving the field in a more suitable state for potato production later in the rotation.

Table 11 Mean nematode populations (eggs/g of soil) pre planting and post harvest

Incorporation method	nematicide	*Pi eggs /g soil	*Pf eggs /g soil	**Pf / Pi
BV	+	81 (4.337)	55 (3.991)	0.70 (0.527)
BV	-	96 (4.403)	76 (4.299)	0.97 (0.661)
BT	+	102 (4.584)	57 (3.983)	0.61 (0.458)
BT	-	88 (4.433)	62 (4.078)	0.74 (0.543)
BDV	+	80 (4.216)	53 (3.840)	0.74 (0.538)
BDV	-	84 (4.298)	62 (4.031)	0.86 (0.599)
BDT	+	81 (4.280)	41 (3.622)	0.54 (0.427)
BDT	-	81 (4.252)	83 (4.355)	1.24 (0.773)
DV	+	79 (4.123)	88 (4.326)	1.26 (0.806)
DV	-	68 (4.176)	61 (4.090)	1.01 (0.675)
DT	+	107 (4.580)	91 (4.390)	0.96 (0.644)
DT	-	73 (4.275)	80 (4.349)	1.15 (0.747)
VV	+	131 (4.865)	74 (4.234)	0.58 (0.445)
VV	-	103 (4.606)	99 (4.573)	1.00 (0.686)
VT	+	65 (4.146)	71 (4.223)	1.18 (0.751)
VT	-	62 (4.074)	57 (3.854)	0.87 (0.609)
S.E.(7D.F.)		17.4 (0.2105)	12.7 (0.2026)	0.180 (0.0876)
C.V%.		45.9 (10.5)	35.8 (9.2)	47.8 (34.1)

* Natural log of data in parentheses

** Natural log+1 of data in parentheses

Table 12 Mean nematode populations and nematicide use

Nematicide	*Pi eggs /g soil	*Pf eggs /g soil	**Pf/Pi
+	91 (4.396)	66 (4.076)	0.82 (0.575)
-	82 (4.315)	76 (4.204)	0.98 (0.662)
S.E.(1D.F.)	6.25 (0.0725)	3.92 (0.0602)	0.0679 (0.0333)
C.V%.	31.4 (7.8)	32.2 (8.8)	29.4 (20.6)

* Natural log of data in parentheses

** Natural log+1 of data in parentheses

4.2.2.8 Population changes in the furrow

Results from soil samples taken from the furrows adjacent to treated beds showed no significant differences between incorporation treatments or nematicide types (Table 13). However, overall the furrows showed a significant reduction in nematode populations ($P \leq 0.05$) with a mean Pf/Pi ratio of 0.59 compared with the reduction in treated beds of 0.82 (Table 14). Several reasons could explain this, the randomisation of the plots for this Experiment may have caused sufficient soil compaction from machinery in the furrows to prevent roots successfully colonising the furrows and therefore the greater reduction in the furrows could be due to natural decline of the pest. Natural decline of PCN generally occurs at a rate of 30% each year and at this could explain the 47% reduction in the furrow populations compared with 27% in the bed populations. However, *G. pallida* has been reported to decline more slowly at approximately 15% per annum (Whitehead, 1993). If this is the case for Field Experiment 1 some other factor may have contributed to the higher rate of decline in the furrow. The higher rate of decline in the furrow could be attributed to

movement of nematicide to the furrows by irrigation or rain water. Oxamyl and aldicarb are soluble in water which tends to accumulate in the furrows after the application of irrigation water or rainfall. Therefore the nematicides could be moved and concentrated in the furrow resulting in a higher rate of PCN decline. However, the short half life of granular nematicides probably means that by the time roots have reached the furrow the nematicide will have degraded, and that the greater decline in furrows is more to do with rooting density.

Table 13. Nematode populations in nematicide treated beds and adjacent furrows.

Incorporation /nematicide	Bed =B Furrow=F	*Pi eggs /g soil	*Pf eggs /g soil	**Pf / Pi
BV	B	81 (4.377)	55 (3.991)	0.70 (0.527)
BV	F	82 (4.340)	40 (3.549)	0.48 (0.385)
BT	B	102 (4.584)	57 (3.983)	0.61 (0.458)
BT	F	106 (4.568)	42 (3.612)	0.40 (0.331)
BDV	B	80 (4.216)	53 (3.840)	0.74 (0.538)
BDV	F	59 (3.955)	36 (3.566)	0.70 (0.525)
BDT	B	81 (4.280)	41 (3.622)	0.54 (0.427)
BDT	F	48 (3.787)	19 (2.944)	0.48 (0.381)
DV	B	79 (4.123)	88 (4.326)	1.26 (0.806)
DV	F	64 (4.009)	54 (3.877)	0.92 (0.643)
DT	B	107 (4.580)	91 (4.390)	0.96 (0.644)
DT	F	87 (4.325)	46 (3.750)	0.59 (0.460)
VV	B	131 (4.865)	74 (4.234)	0.58 (0.445)
VV	F	109 (4.650)	48 (3.682)	0.41 (0.335)
VT	B	65 (4.146)	71 (4.223)	1.18 (0.751)
VT	F	61 (4.030)	41 (3.642)	0.71 (0.528)
S.E.(7D.F.)		17.4 (0.2280)	12.4 (0.2276)	0.138 (0.0717)
C.V%.		26.3 (6.4)	41.1 (10.5)	46.9 (34.5)

* Natural log of data in parenthesis, ** Natural log+1 of data in parenthesis

Table14. Mean populations of PCN in nematicide treated beds and adjacent furrows

	*Pi eggs /g soil	*Pf eggs /g soil	**Pf/Pi
Bed	91 (4.396)	66 (4.076)	0.82 (0.575)
Furrow	77 (4.208)	41 (3.578)	0.59 (0.449)
S.E.(1D.F.)	3.5 (0.0438)	3.5 (0.0637)	0.052 (0.0279)
C.V%.	42.6 (10.9)	42.8 (11)	28.6 (19.7)

* Natural log of data in parentheses

** Natural log+1 of data in parentheses

Large numbers of potato roots produced in the furrow later in the growing season may take advantage of the water collected there after irrigation or rainfall. The partially resistant cultivar Santé could in this case have decreased the population of PCN in the furrow more than in the bed due to the relative concentrations of root, as large quantities of roots were observed in the furrows in this particular Experiment.

4.2.3 Conclusions

Field Experiment 1 has shown that the method of application and incorporation of a granular nematicide has no effect on the yield of the potato cultivar Santé which, due to its low tolerance of PCN (Gurr, 1987) would suffer yield reductions if an incorporation method was unsatisfactory. No differences occurred between oxamyl and aldicarb indicating that the two nematicides were equally effective in this situation. The possible effects of the agronomic inputs such as foliar fertiliser and

irrigation on the Experiment were discussed by Woods *et al.* (1995) (Appendix 1) and may have enabled the crop to tolerate the PCN burden and thereby mask any differences in the incorporation techniques. However, this Experiment is aimed at providing data on nematicide performance in a commercial situation and so any possible interactions between agronomic practices used on this Experiment and the incorporation techniques relate directly to commercial practice.

Differences in yield by tuber size did occur, particularly the yield of smaller tubers in some treatments, but ware grade did not show any differences between incorporation treatments or nematicides.

The method of incorporation did not produce any significant effects on nematode multiplication rates, nor did nematicide or nematicide type. The partial resistance of the cultivar used probably caused a uniform degree of PCN control regardless of nematicide treatment, even though nematicide treatment did improve the yield of treated plots. The resistance to PCN that operates in potatoes only prevents the formation of new cysts, and does not prevent invasion of the roots by nematodes. Therefore, no apparent differences in PCN control could be seen relating to improvement in yield. Multiplication rates of nematodes were significantly lower in furrows than in the treated beds. Hancock (1995) suggested that the furrows could be sources of high nematode multiplication as the furrows are not treated with nematicide if it is incorporated using a stone and clod separator. However, in this Experiment no differences occurred in furrow multiplication rates between incorporation treatments. This may be unique to this particular cultivar and a cultivar with no partial resistance may indeed cause higher multiplication within the furrows. The role of natural decline in PCN populations may account for the reduction of populations in the furrow as compared with the bed. Other speculative explanations for the greater reduction in populations could be the movement of hatching factors from

roots in the bed early on in the season which could trigger a hatch in the furrows before roots have reached the hatched juveniles. More work in this area is needed to assess the effects of root distribution, nematode distribution and nematicide distribution on PCN population dynamics.

The experimental design used was a randomised block split plot. This design is generally more sensitive at picking up differences between split plots (i.e. nematicide treated and untreated) than it is between mainplots (incorporation treatments / nematicide type). This Experiment design may have been less sensitive to any differences that may have occurred between incorporation treatments and it was felt that a randomised block design might be better for future experiments. Overall, the differences in tracer placement seen in Chapter 3 and likely to have been repeated in Field Experiment 1 had no significant effects on the growth of a commercial potato crop or the PCN populations.

4.3 Introduction and preliminary soil sampling for Field Experiment 2

4.3.1 Introduction

Field Experiment 2 was situated at the Hasse Estate's Engine farm, Ely, Cambridgeshire managed by Greens of Soham. This farming company grows a variety of crops on the predominantly peat soil types. Onions, wheat, sugar beet, carrots and especially potatoes are grown in close rotation, which has resulted in a severe PCN problem on many fields. The cultivation of potato ground on the estate uses stone and clod separators to remove clods of clay which make up the sub soil of the area. As the peat has eroded the occurrence of clay in seedbeds has increased and now presents a major problem when potatoes are harvested using unmanned harvesters. The site was chosen because of the soil type, high PCN populations and the use of stone and clod separation in the area.

4.3.2 Method

For preliminary mapping of PCN distribution in the field, twenty 20m x 20m squares covering an area of 0.8 ha were marked out (see Fig. 10). Thirty soil cores (2.5cm x 22.5cm) were taken in a systematic manner from each square and then bulked. Soil samples were analysed using standard methods described for the initial sampling of Field Experiment 1.

4.3.3 Results

The average populations found infesting the proposed Experiment area are shown in Fig. 10.

4.3.4 Comments

From Figure 10 it can be seen that there are two distinct areas of PCN infestation. The western area of the site shows an extremely high average population density in excess of 300 eggs/g of soil. The eastern side of the site closest to the headland had a lower population of around 150 eggs/g of soil. The recommendations for granular nematicide use state that growing potatoes in land infested with PCN populations in excess of 80 eggs/g will show little response to granular nematicide applications (Anon, 1995b). However, I suspect that many growers with PCN infested land are ignoring this advice and I decided to place the Experiment on the eastern side of the site with the aim of investigating the efficacy of granular nematicides in a high population situation, and also to see if the placement of nematicide in this situation exaggerates the problem, particularly if the nematicide is incorporated poorly. Unfortunately the farm manager left an area for the Experiment which fell on both the areas, effectively half on the very high population and half on the lower population.

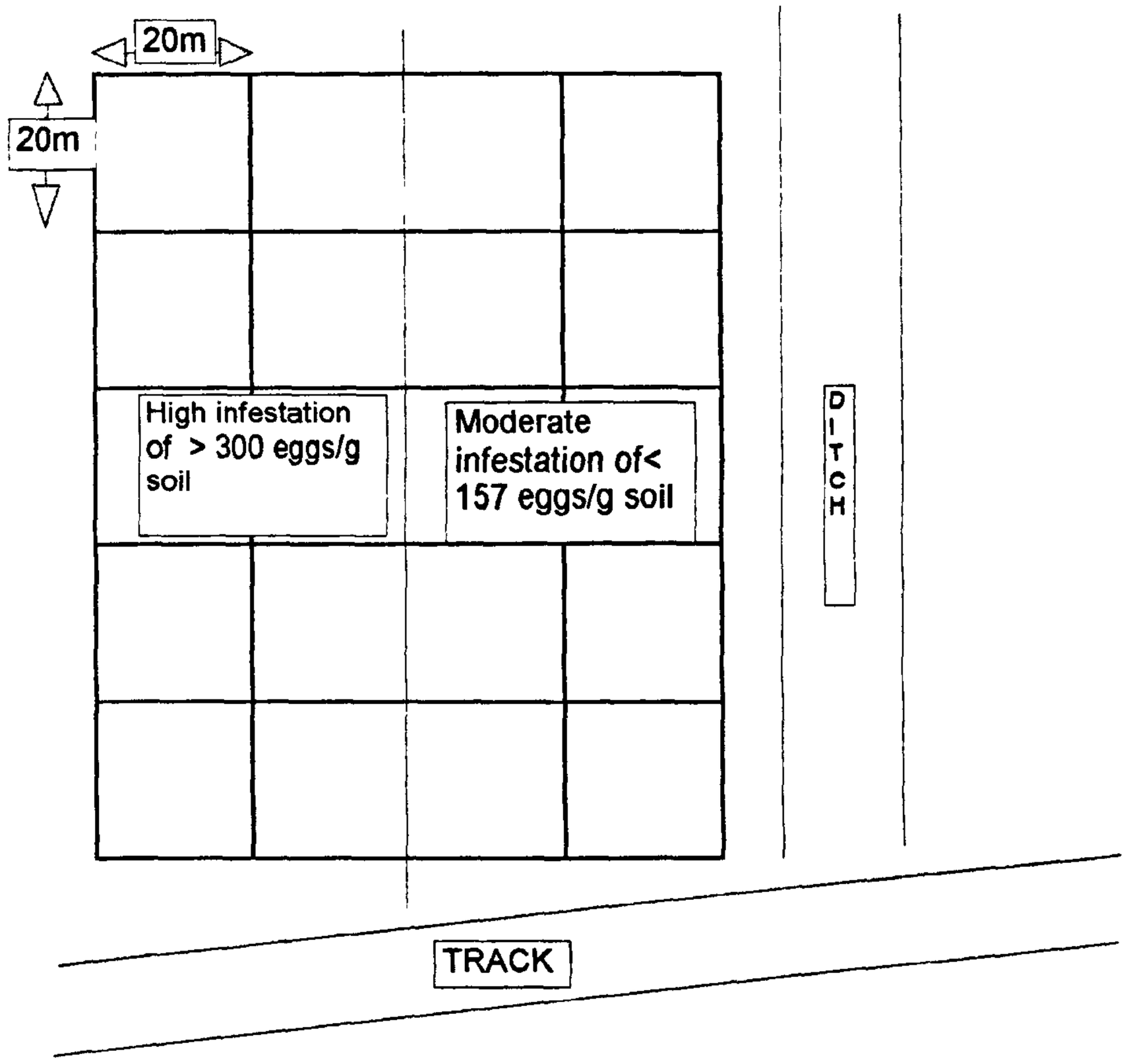


Figure 10. Sampling grid and population characteristics for Field Experiment 2.

4.4 Field Experiment 2: A comparison of techniques used for applying and incorporating granular nematicides for the control of potato cyst nematode *Globodera pallida* on a peat soil

4.4.1 Materials and methods

4.4.1.1 Experimental Design

The experiment was of a randomised block, split plot design. The plot size for this field Experiment was 6m x 10.2m and each treatment was replicated four times (see Fig. 11).

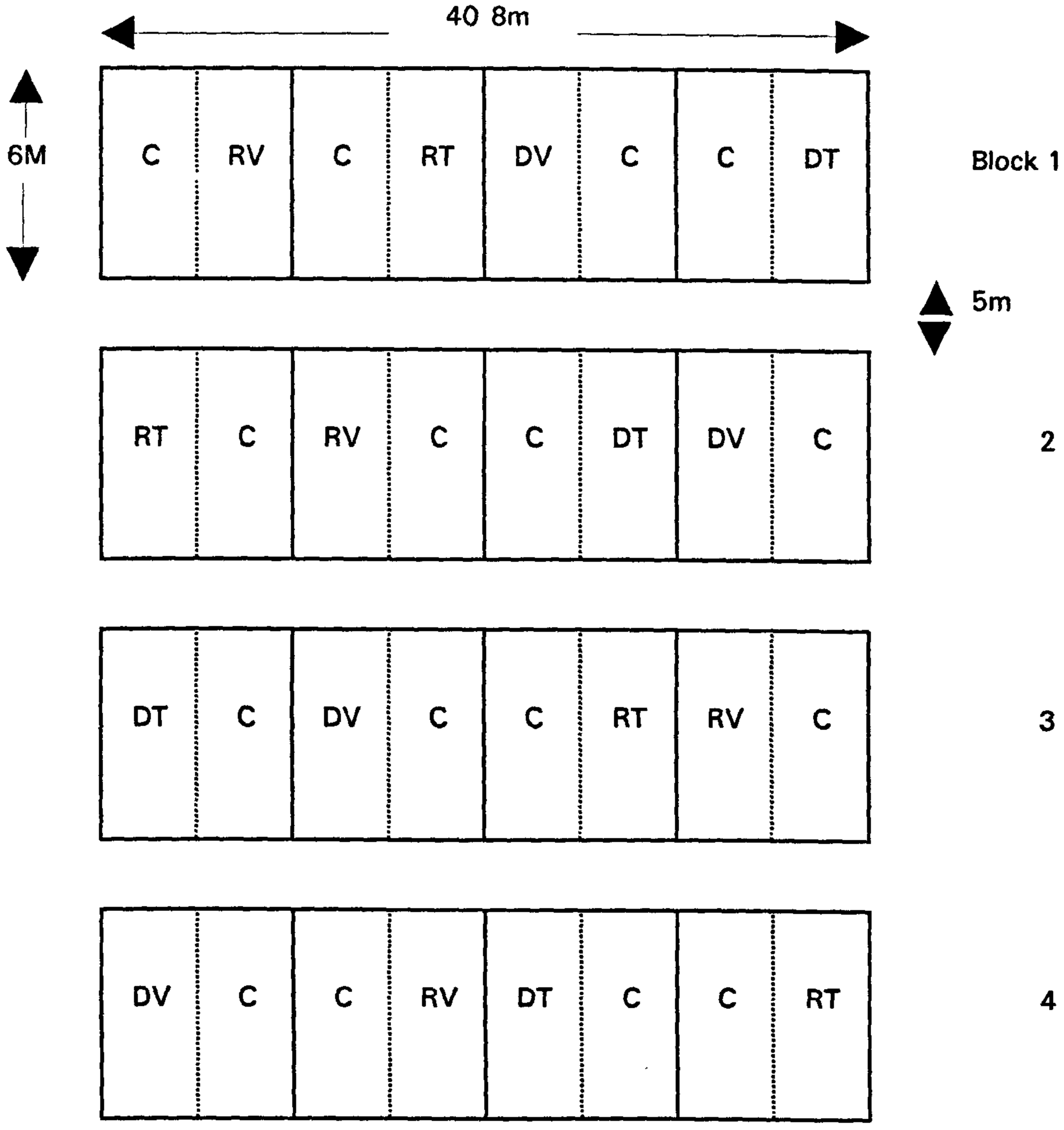
4.4.1.2 Experimental Methods

The experiment was carried out at the Hasse Estate's Engine Farm. Two methods of incorporating the nematicides were compared:

1) Broadcasting nematicide followed by L-blade rotavator incorporation.

2) Pearson Megastar stone and clod separator applying and incorporating nematicide as described in Chapter 2.

It was not planned to use a stone and clod separator with the application of nematicide occurring half way up the machine, as in Chapter 3 this method was found to have serious limitations. However, difficulty in finding a suitable destoner in the Ely area meant that the this particular method of destoner incorporation was the only option. Aldicarb and oxamyl were compared using each incorporation method. The two nematicides were applied as Vydate 10G (oxamyl 10% ww a.i. granules) and Temik 10G (aldicarb 10% ww a.i granules) according to the manufacturers' recommendations for the population densities found in the Experiment area. A control with no nematicide treatment was included for each incorporation method.



C = UNTREATED CONTROL

RV = ROTAVATED VYDATE

RT = ROTAVATED TEMIK

DV = DESTONER VYDATE

DT = DESTONER TEMIK

Figure 11. Plot layout for Field Experiment 2

4.4.1.3 Planting

Plots were mechanically planted with the *G. pallida* susceptible cultivar Fianna on the 11/5/94. Super elite seed tubers were planted 30 cm apart in 90cm rows. Details of crop inputs can be found in Appendix 2.

4.4.1.4 Growth analysis

Six weeks after planting two whole plants were removed from the end meter of each plot and dissected as described for Field Experiment 1.

4.4.1.5 Harvesting

The haulm from the plants in the two centre rows was removed by hand on the 31/8/94. A 5m length of the harvest rows from each plot was then lifted by hand using a fork between the 31/8/94 and the 2/9/94 1994. Tubers were mechanically riddled over 45mm and 65mm webs. Tubers greater than 45mm constituted ware yield.

4.4.1.6 Soil Sampling for Potato cyst-nematode

Bulk samples of 60 cores (1.0cm diameter and 9cm deep) were taken from the middle two rows of each subplot, and also from the furrows adjacent to the middle two treated rows. Row samples were taken immediately after planting and after harvest, furrow samples were taken immediately after planting and immediately before harvest. The number of eggs in them were estimated by standard methods described in Field Experiment 1 methods.

4.4.1.7 Data analysis

Data from the field Experiment were analysed as described for Field Experiment1.

4.4.2 Results and discussion

4.4.2.1 Abbreviations

RV=Rotavator incorporating Vydate
RT=Rotavator incorporating Temik
DV=Destoner incorporating Vydate
DT=Destoner incorporating Temik

4.4.2.2 Six week growth analysis

Table 15. Effect of nematicide treatment on plant dry weight and numbers of PCN juveniles invading the roots of variety Fianna six weeks post planting (27/6/94)

	Plant dry weight(g)					Juveniles
nematicide	haulm	root	stolon	tuber	total	/g root
+	18.8	1.02	0.43	15.4	35.7	935
-	14.7	0.93	0.39	11.6	27.7	1169
SE(1DF)	2.26	0.091	0.073	1.98	4.18	119.5
C.V%.	53.9	37.3	70.3	58.5	52.8	42.9

No significant differences occurred between incorporation treatments and nematicide type at six weeks after planting. Table 15 shows mean plant dry weights for nematicide treated and untreated plots. Nematicide had no significant effect on the plant dry weights. No significant differences occurred in numbers of nematodes in the roots for treated and untreated plots presumably because of poor "kill".

4.4.2.3 Yield

Table 16. Mean yield and nematicide use for Field Experiment 2

	nematicide		
	+	-	S.E (1 D.F)
Total yield t/ha	12.6	10.6	0.68
Ware yield t/ha	7.3	6.5	0.40
Percentage ware	38.6	35.8	2.24

Mean yields for nematicide treated and untreated plots are presented in Table 16. Mean yields for this Experiment were extremely poor with nematicide treated plots only achieving 12.6t/ha and untreated plots 10.6t/ha. No significant differences occurred between incorporation treatments and nematicide type. Total, ware and percentage ware yields were higher in nematicide treated plots, but this was not statistically significant or economically important with a mean increase in ware yield of 0.82 t/ha.

Table 17. Nematicide use and tuber yield by grade for Field Experiment 2

Tuber yield by grade	Nematicide		
	+	-	S.E.(1 D.F.)
<45mm t/ha	5.3	4.1	0.46
45-65mm t/ha	7.1	6.3	0.39
65+mm t/ha	0.2	0.2	0.03

Table 17 shows the mean tuber yields by grade for treated and untreated plots. Tuber yields for all grade fractions were higher in nematicide treated plots but the higher yields were not significantly different from those in untreated plots.

4.4.2.4 Nematode population estimation

4.4.2.5 Within beds population changes

Table 18 shows the mean nematode populations pre planting and post harvest for Field Experiment 2. The mean Pi values are extremely high with an average Pi for the site of 345 eggs/g of soil. Such high populations were not intended for this Experiment but unfortunately, the intended site and actual site did not coinside as the farm manager left an area of ground for the Experiment in the middle of a patch previously determined to be of very high and variable populations. This highlights the difficulty of conducting remote field experiments where liaison with the farmer is limited to the telephone, which is generally only used at the last moment leaving little time for action.

Pf values and the corresponding PF/PI values showed little change from the initial populations. This is probably due to the high competition for nematode feeding sites that would occur at high initial population densities. No significant differences occurred between the incorporation treatments or nematicide type.

The mean nematode populations for treated and untreated plots are shown in Table 19. The nematicides used had no significant effect on nematode multiplication rates, and the stability of the population as shown in the Pf/Pi values is again likely to be a consequence of high intraspecific competition. The high Pi for this Experiment is well above the recommended limit of 80 eggs/g of soil for nematicide use and it is therefore not surprising that the yields were low and nematicide use provided little benefit for nematode control.

Table 18. Mean nematode populations (eggs/g soil) preplanting and post harvest.

Incorporation method	nematicide	*Pi eggs / g of soil	*Pf eggs / g of soil	**Pf / Pi
RV	+	376 (5.824)	389 (5.952)	1.03 (0.710)
RV	-	346 (5.777)	281 (5.548)	0.81 (0.594)
RT	+	360 (5.650)	311 (5.736)	0.86 (0.623)
RT	-	425 (5.874)	355 (5.835)	0.84 (0.607)
DV	+	416 (5.751)	275 (5.98)	0.66 (0.507)
DV	-	203 (5.124)	321 (5.749)	1.58 (0.948)
DT	+	311 (5.013)	252 (5.521)	0.81 (0.593)
DT	-	326 (5.420)	359 (5.861)	1.10 (0.743)
MEAN		345(5.845)	317(5.762)	0.92(0.652)
S.E. (3D.F)		158.1 (0.4629)	44.4 (0.1433)	0.836 (0.2431)
C.V%.		35.1 (7.1)	20.2 (3.7)	83.9 (33.7)

*Natural log shown in parentheses

**Natural log+1 shown in parentheses

Table 19. Mean nematode populations and nematicide use

Nematicide	*Pi eggs /g soil	*Pf eggs /g soil	**Pf/Pi
+	366 (5.903)	307 (5.727)	0.84 (0.610)
-	325 (5.784)	329 (5.796)	1.01 (0.698)
S.E.(1D.F.)	30.4 (0.984)	16.0 (0.0531)	0.339 (0.716)
C.V%.	35.1 (7.1)	20.2 (3.7)	83.9 (33.7)

*Natural log shown in parentheses

**Natural log+1 shown in parentheses

4.4.2.6 Population changes between the bed and furrow

The results from the soil analysis from the furrows adjacent to treated beds showed no significant differences between incorporation treatments or nematicide types. However, overall the furrows showed a significant reduction in nematode populations ($P \leq 0.05$) with a mean Pf/Pi ratio of 0.28 compared with the reduction in treated beds of 0.84 (Table 20). Again the same reasons discussed for Field Experiment 1 could explain the high decline of PCN in the furrows. However, the cultivar Fianna which was used in this Experiment is susceptible to *G.pallida* and therefore the theory of the partially resistant roots present in large quantities in the furrows does not hold for this Experiment. This leaves the natural decline which is also unlikely as the populations in the furrows of Experiment 2 have declined by 72%. High populations of PCN may decline at a faster rate than small populations (Whitehead, 1980). However, population decline occurs in the absence of a host, and potato roots were present in the furrows in this experiment.

Table 20. Nematode populations in nematicide treated beds and adjacent furrows

	Pi eggs /g soil	Pf eggs /g soil	Pf/Pi
Bed	366	307	0.84
Furrow	310	87	0.28
S.E.(1D.F.)	28.2	19.1	0.356
C.V.	33.4	38.7	141.1

Another explanation could involve a phenological type approach: roots growing into the furrows later in the season may induce a hatch and subsequently be invaded by juveniles, but these juveniles may be unable to complete the life cycle if invasion occurs close to harvest. This Experiment was harvested in late August and consequently a trap cropping effect may have been responsible for the observed decline in the furrows.

4.4.3 Conclusions

From the data presented the two methods of incorporation used, rotavator and stone and clod separator, had no effect on the growth and subsequent yield of the potato cultivar Fianna. The two nematicides Temik and Vydate also had no effect on the growth and yield of the crop. The initial nematode populations found on this Experiment were too high to be able to make any sound judgements about the incorporation of granular nematicides, and the variability of the initial populations makes analysis of the experiment very difficult. The Pi's found on the site ranged from 32 to 943 eggs/g of soil. The site, as shown in the initial sampling for this

Experiment, was unfortunately positioned in such a way that half the Experiment was on a very high PCN infestation and the other half on a moderate to high infestation. Analysis of the two separate areas yielded no new information, the overall effects of nematicide incorporation and nematicide type were similar to those presented in this chapter.

The variation in initial egg counts was used to calculate a regression of ware yield against the initial PCN population. This would give an indication of the cultivars tolerance to PCN in this situation. However, from the regressions it was obvious that any tolerance that this cultivar possessed lies somewhere below a threshold of 32 eggs/g of soil. The breeders information on the cultivar Fianna describes it as drought tolerant, but poor yields of <20t/ha for the lowest Pi of 32 eggs/g certainly shows little tolerance to water stress be it caused by decreased root efficiency by PCN invasion or low rainfall.

A significant reduction in furrow populations compared with the adjacent bed populations occurred in this Experiment. The decrease in populations was greater than that which occurred in Field Experiment 1. As discussed, a combination of natural wastage early in the growing season followed by a trap crop effect at harvest is the likely cause of this reduction.

Overall the differences seen in incorporation of tracer in Chapter 3 did not have an effect on crop growth or PCN population dynamics. Future field experiments situated on commercial farms will require closer liaison with the host farmer in order to ensure that the best possible site is obtained.

5.0 CHAPTER 5

FIELD EXPERIMENTS 3 AND 4

5.1 Introduction and preliminary soil sampling for Field Experiment 3 1995

5.1.1 Introduction

Field Experiment 3 was situated on Messrs Brian and Gordon Maddocks' farm at Great Bolas, Shropshire. The farm has a history of PCN problems with the species *G. pallida* predominating. According to the farm records, the experiment site selected for the 1995 experiment had a previous population density of approximately 40 eggs/g of soil.

5.1.2 Methods

The field was intensively sampled for PCN population density estimation using the method described in Chapter 4.

5.1.3 Results

Figure 12 shows the results from the initial sampling survey.

5.1.4 Comments

From Fig. 12 it can be seen that the distribution of PCN within the field was uneven. The population on half of the field was generally low but on the western half it ranged from moderate to high. This conflicted with the farmer's view that the whole of the area sampled was highly infested, particularly with respect to the lightly infested south eastern area of the field. This demonstrates the limitations of whole field sampling for PCN due to the variability of populations within a field and highlights the need for more intensive automated sampling techniques to be developed and adopted (Haydock and Evans, 1994). The area chosen for the experiment was within grid numbers M36, 37, 1, 2 6 and 7 giving an average moderate to high infestation of 55 eggs / g of soil.

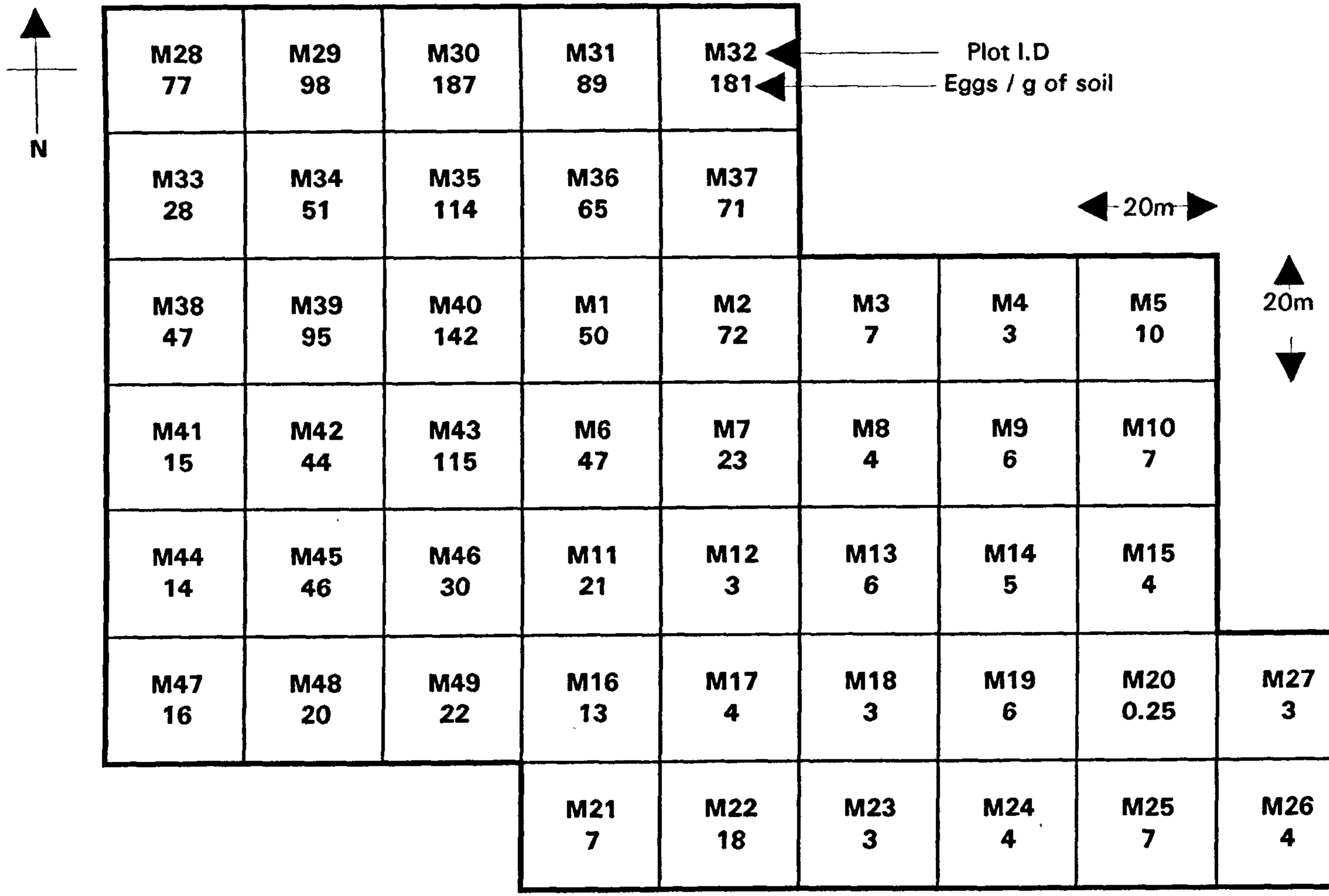


Figure 12. Initial sampling grid and associated PCN population densities for Field Experiment 3

5.2 Field Experiment 3: Application of the granular nematicide oxamyl pre, post or during stone and clod separation for the control of the potato cyst nematode *Globodera pallida* with subsequent measurement of the yield of the potato cultivar Maris Piper.

5.2.1 Objectives

Field Experiment 3 was undertaken to investigate the three timings of nematicide incorporation that are feasible using a bed tiller and stone and clod separation in order to concentrate on the most popular methods of granular nematicide incorporation used by growers.

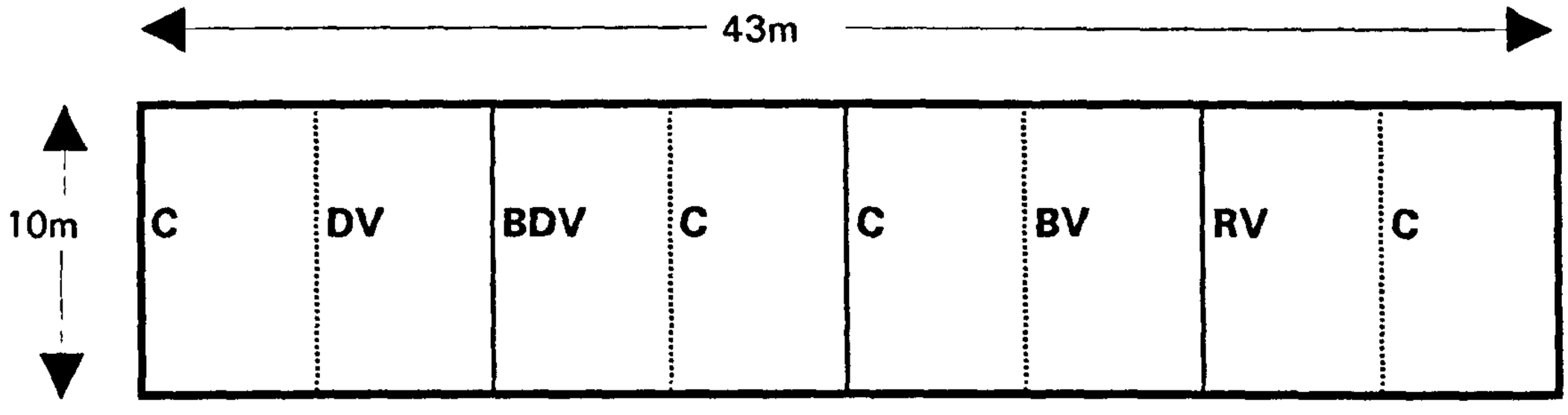
5.2.2 Materials and methods

5.2.2.1 Experimental design

This field experiment was designed as a randomised block split plot. The split plot size was 10 x 5.4m each plot comprising six rows of potatoes replicated 5 times (Fig. 13)

5.2.2.2 Application and incorporation of oxamyl

The granular nematicide Vydate 10G (oxamyl 10% ww a.i. granules) was applied before, after or during stone and clod separation. The standard broadcast rotavation technique was used as a comparison to the other treatments as follows:



C= UNTREATED CONTROL
BV= PLOT DESTONED, THEN BED TILLER TO INCORPORATE VYDATE
BDV=BED TILLER TO INCORPORATE VYDATE, THEN DESTONE
RV=BROADCAST VYDATE,THEN ROTAVATE
DV=DESTONER APPLYING AND INCORPORATING VYDATE

Figure 13. Plot layout for Field Experiment 3

Application and incorporation before stone and clod separation: A bed tiller as described in Chapter 2 was used to apply and incorporate the nematicide prior to stone and clod separation. The stone and clod separator made a pass after the bed tiller to give a second incorporation of the nematicide.

Application and incorporation during stone and clod separation: A Grimme Colt stone and clod separator as described in Chapter 2 was used to apply and incorporate Vydate.

Application and incorporation after stone and clod separation: A bed tiller was used to apply and incorporate oxamyl to plots after they had been destoned.

Spiked rotavator: Plots which were to be treated using the standard broadcast rotavation method were completely levelled using a fork to simulate a flat ground situation prior to nematicide incorporation. A spiked rotavator as described in Chapter 2 was used to incorporate Vydate into the level ground.

5.2.2.3 Planting

Plots were mechanically planted with the cultivar Maris Piper on the 5/4/95. Super elite seed tubers were planted 30 cm apart in 90cm wide rows. Details of crop agronomic inputs can be found in Appendix 2.

5.2.2.4 Percentage ground cover

The percentage ground cover from each plot was measured weekly from 26/5/95 to 3/7/95 using the method described in Chapter 4.

5.2.2.5 Growth analysis

Six weeks after planting, 2 whole plants were removed from the end 1m of each plot, dissected and the information obtained as described in Chapter 4.

5.2.2.6 Harvesting

The haulm from the plants in the two centre rows was removed on the 3/9/95. A 5m length from each of the two harvest rows was then lifted by hand using a fork between 4th and 6th of September 1995. Tubers were mechanically riddled over 45mm and 65mm webs. Tubers greater than 45mm constituted ware yield.

5.2.2.7 Soil sampling for potato cyst-nematode

Bulk samples of 60 cores (1.0cm diameter and 9cm deep) were taken from the middle two rows of each subplot before planting and after harvest. The numbers of eggs in them were estimated by standard methods described in Chapter 4.

5.2.2.8 Data analysis

Data from Field Experiment 3 were analysed using Genstat 5 version 4.

5.2.3 Results and discussion

5.2.3.1 Six week growth analysis

Table 21 shows the effect of nematicide incorporation treatment on plant growth 6 weeks after planting. No significant differences occurred between treatments. This suggests that the method of applying and incorporating a granular nematicide had no effect on plant growth up to six weeks after planting. The total root invasion of plants by PCN juveniles when the bed tiller incorporated Vydate was lower than the other treatments, but was not significantly different. The lower root invasion for this treatment did not cause any corresponding effect on plant growth. The number of plants taken per plot for destructive analysis in this experiment was 2. This may not be sufficient to gain a realistic view of treatment variation. However, increasing the sensitivity of the destructive analysis by taking more plants per plot was not possible as it exceeded the resources available for this experiment.

Table 22 shows the effect of nematicide on plant growth and nematode invasion six weeks post planting. Plant growth responded significantly to the use of a granular nematicide, with haulm, tuber and total plant dry weights all significantly higher ($P \leq 0.05$) for nematicide treated plots than untreated plots.

Tuber numbers and total root invasion by PCN juveniles were also significantly higher ($P \leq 0.05$) for nematicide untreated plots than treated plots. This suggests that Vydate probably improved the growth of treated plants because less nematodes invaded the root system, resulting in a healthier root system and increased water and nutrient uptake.

Table 21. Effect of nematicide incorporation treatment on plant growth six weeks post planting

Incorporation method	nematicide	Plant dry weight (g)				tuber no.	Juveniles /g root
		haulm	roots	tubers	*Total		
Rotavator	+	35.50	1.74	8.02	46.10	20	527
	-	31.40	2.16	4.64	38.90	18	1521
Bed tiller	+	38.10	1.76	8.28	49.20	23	46
	-	24.10	1.90	3.32	29.90	12	1624
Bed tiller / destoner	+	38.40	2.01	7.92	49.30	21	761
	-	28.40	1.89	5.93	36.70	16	1251
Destoner	+	41.70	2.10	7.87	52.80	21	413
	-	29.40	2.15	5.20	37.50	20	1306
S.E. (3D.F.)		3.510	0.254	1.223	4.510	2.2	280.6
CV%		20	28	38	19	20	68

*Total includes dry stolon weight data

Table 22. Effect of nematicide on plant growth six weeks after planting

	Plant dry weight (g)				Tuber no.	Juveniles
nematicide	haulm	root	tuber	Total*		/g root
+	38.4	1.9	8.0	49.4	21	437
-	28.3	2.0	4.8	35.8	16	1425
S.E. (1D.F.)	1.46	0.12	0.55	1.83	0.8	141.0
C.V%.	18	19	31	18	21	44

*Total includes dry stolon weight data

5.2.3.2 Crop percentage ground cover

Table 23. Nematicide use and mean percentage ground covers for Field Experiment 3

Percentage	nematicide		
ground cover	+	-	S.E.(1D.F.)
26/5/95	11.7	9.2	0.84
5/6/95	34.9	27.6	1.19
12/6/95	61.5	47.9	0.95
19/6/95	75.6	61.1	1.16
26/6/95	90.9	74.1	1.21
3/7/95	99.1	86.3	1.51

No significant differences occurred in percentage ground cover between nematicide incorporation treatments; therefore, means for nematicide

treated and untreated plots are presented in Table 23. Percentage ground cover was significantly higher ($P \leq 0.05$) for nematicide treated plots than for untreated plots on all but the first sampling dates. As a potato plant becomes more autotrophic after emergence, its sensitivity to environmental factors such as water supply and mineral nutrients increases (Moorby, 1978). The first percentage ground cover measurements were made approximately seven weeks after planting, so residual nutritional effects from the mother tuber may have disguised any effects on growth caused by nematodes invading the root system. Later in the growing season, marked differences in plant ground cover occurred with nematicide treated plots having higher percentage ground cover than untreated plots, probably as a result of the healthier root systems produced by nematicide treatment allowing greater uptake of nutrients and water for plant growth.

5.2.3.3 Yield

From Table 24 it can be seen that no significant differences occurred in the mean yields for nematicide incorporation treatments. Total yield, ware yield and percentage ware yield were similar for all the incorporation methods used. Table 25 shows the mean yields for nematicide treated and untreated plots. Nematicide treatment significantly increased ($P \leq 0.05$) total yield by 9.5 t/ha and ware yield by 9 t/ha. Percentage ware yield was also significantly increased by nematicide treatment, but only by a small margin of 2.5%.

Table 24. Mean yields for Field Experiment 3

Incorporation method	nematicide	total yield (t/ha)	ware yield (t/ha)	percentage ware
Rotavator	+	57.5	48.1	83.9
	-	44.7	36.4	81.0
Bed tiller	+	55.3	45.1	81.4
	-	45.4	36.0	79.0
Bed tiller / destoner	+	54.7	45.9	83.5
	-	45.9	36.8	80.3
Destoner	+	55.7	45.8	82.2
	-	49.0	39.7	80.7
S.E. (3D.F.)		1.65	1.87	1.63
C.V%.		8	9	3

Table 25. Mean yield and nematicide use for Field Experiment 3

	nematicide		
	+	-	S.E (1D.F.)
Total yield t/ha	55.8	46.3	0.87
Ware yield t/ha	46.3	37.3	0.79
Percentage ware	82.7	80.2	0.56

Tuber yield by grade for nematicide incorporation treatments is shown in Table 26. No significant differences occurred between incorporation methods, with <45mm, 45-65mm and 65+mm tuber grades showing similar yields for each treatment. However, when means are presented for nematicide treated and untreated plots significant differences ($P \leq 0.05$) as seen with nematicide treated plots producing higher 45-65mm and 65+mm tuber yields than untreated plots. The small <45mm grade showed a 0.5 t/ha increase in yield for nematicide treated plots, but this was not statistically different to untreated plots (Table 27).

Table 26. Tuber yield by grade for Field Experiment 3 (t/ha)

Incorporation method	nematicide	<45mm	45-65mm	65+mm
Rotavator	+	9.1	41.5	6.9
	-	8.3	31.1	5.3
Bed tiller	+	10.2	36.4	8.7
	-	9.3	30.7	5.4
Bed tiller / destoner	+	8.9	39.6	6.3
	-	9.1	31.0	5.8
Destoner	+	9.9	39.1	6.6
	-	9.3	34.0	5.8
S.E. (3D.F.)		0.72	1.73	0.99
C.V%.		13	9.5	23

Table 27. Nematicide use and tuber yield by grade for Field Experiment 3

	nematicide		
	+	-	S.E (1D.F.)
<45mm t/ha	9.5	9.0	0.28
45-65mm t/ha	39.2	31.7	0.76
65+mm t/ha	7.1	5.6	0.33

5.2.3.4 Nematode population estimation

The initial population densities (Pi), final population densities (Pf) and population changes (Pf/Pi) are shown in Table 28. No significant differences were found between the Pi's in this experiment but the initial population densities of plots treated with nematicide and incorporated using a spiked rotavator were lower than in other treatments. The variability of the initial population densities found on this experiment site is less than that for Field Experiments 1 and 2 but is still high enough to warrant the use of analysis of covariance for data presented in this chapter. Again, no significant differences in final population density and population change occurred between treatments. Incorporation by a bed tiller and by rotavation produced lower Pfs than those achieved by stone and clod separation and bed tiller followed by stone and clod separation. However, these differences were not statistically different. The cultivar Maris Piper has no resistance to *G. pallida,* so any reductions in population multiplication are likely to be as a result of nematicide treatment.

Table 29 shows the mean population changes that occurred in nematicide treated and untreated plots. Overall the initial population densities were similar for nematicide treated and untreated plots. Final population densities were significantly higher ($P \leq 0.05$) in untreated plots with a

mean Pf of 376 eggs/g of soil compared with a mean population density of 143 eggs/g of soil in treated plots. Overall the population density in untreated plots increased significantly ($P \leq 0.05$) with a Pf/Pi value of 10.59, almost three times the increase found on nematicide treated plots.

Table 28. Mean nematode populations (eggs/g of soil) pre planting and post harvest

Incorporation method	nematicide	*Pi eggs/g soil	*Pf eggs/g soil	*Pf/Pi eggs/g soil
Rotavator	+	41(3.447)	109(4.592)	3.71(1.148)
	-	53(3.797)	380(5.869)	11.16(2.073)
Bed tiller	+	79(4.181)	128(4.815)	2.25(0.635)
	-	72(4.191)	349(5.849)	5.82(1.659)
Bed tiller / destoner	+	51(3.749)	172(5.111)	4.56(1.364)
	-	37(3.436)	431(6.034)	16.93(2.594)
Destoner	+	64(4.000)	164(5.078)	3.66(1.082)
	-	63(3.934)	346(5.814)	8.45(1.888)
S.E. (3D.F.)		13.0(0.2709)	37.3(0.1586)	2.439(0.2923)
C.V%.		37(12)	32(6)	73(36)

*Natural log of data in parentheses

Table 29. Mean nematode populations and nematicide use

Nematicide	*Pi eggs/g soil	*Pf eggs/g soil	*Pf/Pi eggs/g soil
+	59(3.844)	143(4.899)	3.55(1.058)
-	57(3.839)	376(5.892)	10.59(2.054)
S.E.(1D.F.)	4.8(0.1063)	18.3(0.0777)	1.152(0.1243)
C.V%.	37(12)	23(5)	57(34)

*Natural log of data in parentheses

5.2.4 Conclusions

Field Experiment 3 showed that the method of incorporating the granular nematicide Vydate had no effect on plant growth or tuber yield of the potato cultivar Maris Piper. The experiment also showed that the method of nematicide incorporation has no effect on population change of *G. pallida*. The use of a granular nematicide significantly improved the growth and subsequent yield of the potato cultivar Maris Piper and also gave some control of *G. pallida*. The cultivar Maris Piper is considered to be tolerant of infestation by PCN (Evans and Haydock, 1990) and this may be responsible for the apparent uniformity of yield protection provided by the various methods of nematicide incorporation. The use of a split plot experiment design may not have been appropriate for this type of experiment, and the use of a factorial blocked design is therefore used in Field Experiment 4. The tolerance exhibited by Maris Piper could be expected to compensate for infestation by PCN in low to moderate infestations, but in this experiment the infestations were moderate to high, with the highest mean plot Pi of 79 eggs/g of soil bordering on the upper

threshold for nematicide use in current commercial practice (T. Dawkins, *pers. comm.*). A response to a nematicide may be seen in higher infestations when Maris Piper is grown. Responses were seen in Experiment 3 but not between incorporation methods, only between nematicide treated and untreated plots. Therefore differences in PCN control could occur without corresponding differences in yield or yield components as this cultivar's tolerance may compensate even in high infestations. The differences in PCN control shown by the different incorporation methods follow the conclusions drawn from the fluorescent tracer granule work described in Chapter 3. The use of a rotavator or bed tiller for nematicide incorporation, which produced an even distribution of tracer in the top 20cm of the planted bed, would be expected to have a beneficial effect greater than the double incorporation method. Pf values for nematicide incorporation by the rotavator and bed tiller were lower than those of the double incorporation method and the stone and clod separator.

Overall the results from Field Experiment 3 agree with those found in the previous field experiments: using a nematicide on PCN infested ground whatever the incorporation technique, has a greater effect on potato growth and PCN control than the nematicide incorporation technique itself. Subtle differences between incorporation methods may occur, but they have not been found to be significant.

5.3 Introduction and preliminary soil sampling for Field Experiment 4

5.3.1 Introduction

This field experiment was situated in the Isle of Axholme, South Yorkshire at Polybell Farms Ltd, Bull Hassock Farm. The arable farm manager Mr Phillip Taylor has a keen interest in the management of PCN on the estate. A dedicated experiment site has been set aside for the purpose of studying management techniques such as trap cropping which can be implemented on the rest of the estate if successful. The experiment site of approximately 1 ha was situated on a highly organic sand soil and had been divided into three sections (Fig.14). Two seasons ago, all sections had been trap cropped in order to reduce a high infestation of approximately 250 eggs/g of soil. The following year section 1 was trap cropped again, section 2 was sown with red beet and section 3 was planted with the potato cultivar Record.

5.3.2 Methods

The area was intensively sampled before setting up Field Experiment 4 except for area 3, which was waterlogged at the time. The sampling procedure followed methodology described in Chapter 4

5.3.3 Results

Figure 14 shows the results from the preliminary soil survey of the experiment site. The trap cropping seems to have been quite successful with an average egg count of 18 eggs/g of soil. The effect of the second trap crop would seem to have been negligible with an average egg count of 17 eggs/g of soil for the double trap crop treatment (area 1) and 19 eggs/g of soil for the single trap crop treatment (area 2).

5.3.4 Comments

Grid areas Y5, 6,7,9,10,11,13,14,15,17,18 and 19 were chosen to provide the site for Field Experiment 4. This gave an overall low to moderate egg count of 23 eggs/g of soil, which would permit high multiplication of the populations and therefore give the best chance of any differences between treatments on nematode control to be observed.

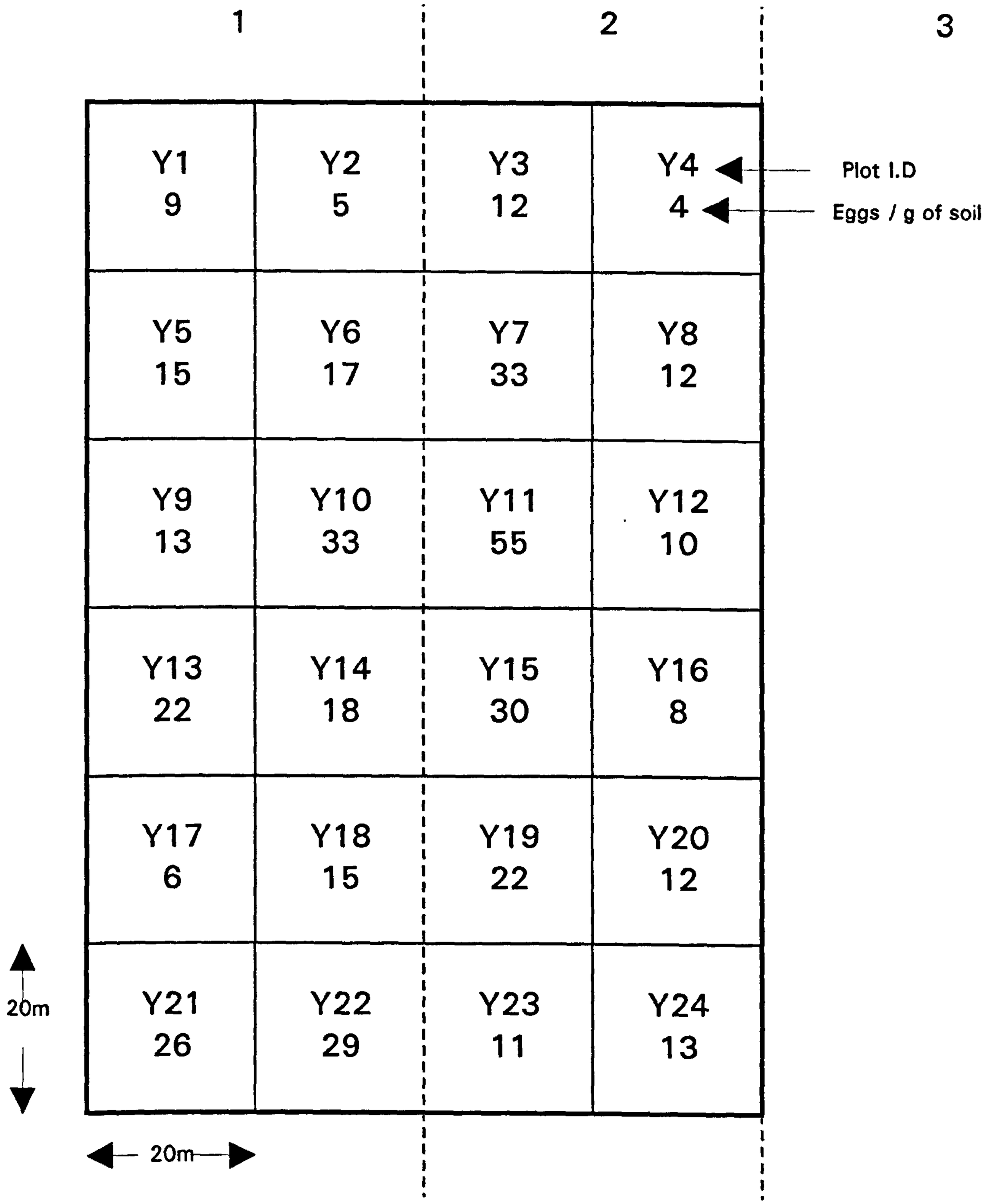


Figure 14. Diagram of initial sampling grid and associated population densities of *G. pallida* for Field Experiment 4.

5.4 Field Experiment 4: Application of the granular nematicide oxamyl pre, post or during stone and clod separation for the control of the potato cyst nematode *Globodera pallida* with subsequent measurement of the yield of the potato cultivar Cara.

5.4.1 Objectives

The objectives of Field Experiment 4 were to assess the effectiveness of the most popular combinations of stone and clod separation and bed tilling for incorporating the granular nematicide Vydate. The potato cultivar Cara was used in order to monitor the effect of nematicide incorporation on this widely grown cultivar. Due to Cara's high level of tolerance to PCN infestation, irrigation and fertiliser were withheld from the trial in order that the crop suffered some stress in this low to moderate infestation.

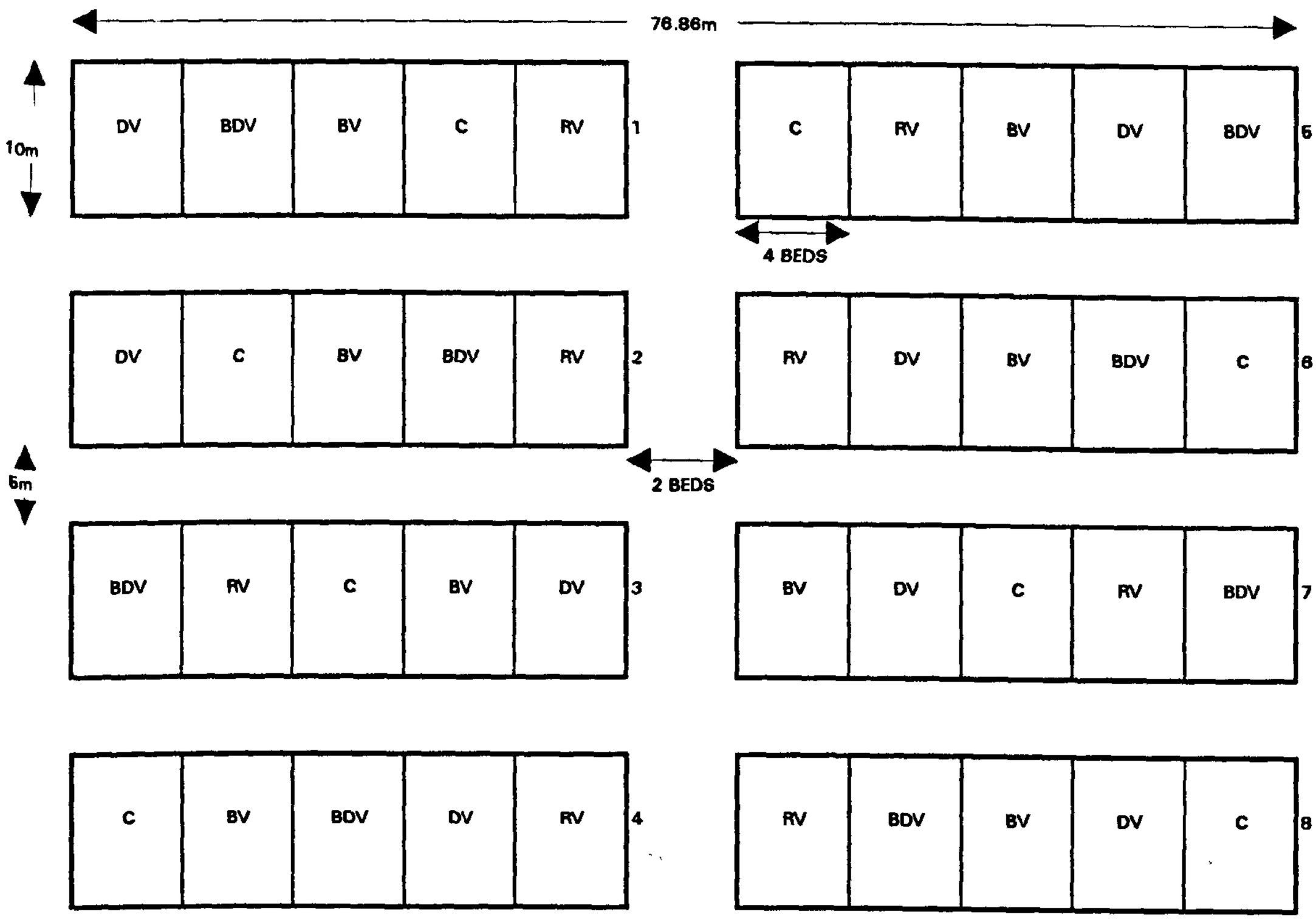
5.4.2 Materials and methods

5.4.2.1 Experimental design

This field experiment was designed as a randomised block. The plot size was 10 x 7.2m comprising eight rows of potatoes replicated 8 times (Fig. 15).

5.4.2.2 Application and incorporation of oxamyl

The granular nematicide oxamyl (Vydate 10G) was applied before, after and during stone and clod separation using the methods descibed below. Also the standard broadcast rotavation technique was used for comparison with the other treatments.



BDV = BEDTILLER FOLLOWED BY DESTONER
BV = DESTONER FOLLOWED BY BED TILLER
DV = DESTONER
C = UNTREATED
RV = BROADCAST THEN ROTAVATED

Figure 15. Plot layout for Field Experiment 4

Application and incorporation of oxamyl before stone and clod separation: A bed tiller consisting of a spiked rotavator with ridging bodies mounted behind the machine was used to incorporate the nematicide prior to stone and clod separation. This machine was fitted with a Horstine Farmery granule applicator calibrated to apply oxamyl at the recommended rate of 5.5kg a.i / ha for the soil type. The nematicide was applied directly in front of the tines whilst the machine was in operation. The stone and clod separator made a pass after the bed tiller to give a second incorporation of the nematicde.

Application and incorporation during stone and clod separation: The stone and clod separator used was a Grimme Mustang custom-fitted with Horstine Farmery applicators calibrated to apply oxamyl at the recommended rate of 5.5 kg a.i / ha.

Application and incorporation after stone and clod separation: The bed tiller was used to apply and incorporate oxamyl in plots after they had been destoned.

Spiked rotavator: Plots which were to be treated using the standard broadcast rotavation method were completely levelled using a fork to simulate a flat ground situation prior to nematicide incorporation. The spiked rotavator was custom-fitted with a Horstine Farmery granule applicator calibrated to apply oxamyl at the recommended rate. The nematicide was applied directly in front of the tines whilst the machine was in operation.

5.4.2.3 Planting

Plots were mechanically planted with the cultivar Cara on the 16/5/95. Seed tubers were planted 30 cm apart in 90cm wide rows. Details of crop agronomic inputs can be found in Appendix 2.

5.4.2.4 Percentage ground cover

The percentage ground cover from each plot was measured once on the 11/7/95 using the method described in Chapter 4.

5.4.2.5 Growth analysis

Eight weeks after planting, 2 whole plants were removed from the end 1m of each plot. Plants were dissected and information gathered as described in Field Experiment 3.

5.4.2.6 Harvesting

The haulm from the plants in the two centre rows was removed on the 18/9/95. A 5m length from each of the two harvest rows was then lifted by hand using a fork on the 19th of September 1995. Tubers were mechanically riddled over 45mm and 65mm webs. Tubers greater than 45mm constituted ware yield.

5.4.2.7 Soil sampling for potato cyst-nematode

Bulk samples of 60 cores (1.0cm diameter and 9cm deep) were taken from the middle two rows of each subplot before planting and after harvest. The number of eggs in them were estimated by standard methods.

5.4.2.8 Data analysis

Data from Field Experiment 4 were analysed using Genstat 5 version 4.

5.4.3 Results and discussion

5.4.3.1 Eight week growth analysis

Table 30. Effect of nematicide incorporation technique on plant growth eight weeks post planting

Incorporation method	Plant dry weight (g)					tuber no	Juveniles /g root
	haulm	root	stolon	tuber	total		
Rotavator	29.00	1.48	0.36	1.97	32.8	6.08	252
Bed tiller	31.80	1.50	0.46	1.23	35.0	5.83	209
Bed tiller/destoner	32.10	1.53	0.33	1.57	35.6	6.25	282
Destoner	28.90	1.49	0.34	1.77	32.5	5.95	236
Untreated control	24.90	1.22	0.31	1.50	27.9	4.89	408
S.E. (4 D.F.)	2.620	0.148	0.056	0.492	3.04	1.082	47.9
C.V%.	25	29	44	86	26	53	49

Table 30 shows the results from plant destructive sampling eight weeks after planting. No significant differences occurred between treatments, but the untreated control generally produced plants which had lower dry weights for haulm, roots, stolons and tubers than the nematicide treatments. The total root invasion for the untreated control is higher than the nematicide treated plots, which may explain the lower plant dry weights. Tuber numbers produced by the different treatments did not differ significantly. The destructive sampling procedure was undertaken eight weeks after planting and the resultant plant dry weights and root invasion

data may be similar statistically for all treatments as, by eight weeks after planting, the soil concentration of the nematicide will have greatly declined. This would perhaps allow nematode invasion to occur on a greater scale in nematicide treated plots with a corresponding decrease in plant weight gain due to lower nutrient and water uptake.

5.4.3.2 Yield

Table 31. Mean yields for Field Experiment 4

Incorporation method	Total yield (t/ha)	Ware yield (t/ha)	Percentage ware
Rotavator	17.7	15.6	88.2
Bed tiller	21.8	20.1	91.3
Bed tiller/destoner	19.0	16.9	88.6
Destoner	19.2	17.3	89.3
Untreated control	20.7	19.1	92.2
S.E. (4 D.F.)	1.61	1.61	1.22
C.V%.	23	26	4

From Table 31 it can be seen that no significant differences occurred between incorporation methods or use of nematicide. Incorporation of Vydate by rotavation produced the lowest total and ware yields, but these were not significantly lower than the other treatments. The variety used for this experiment Cara is tolerant of PCN infestation (Evans and Haydock, 1990), and could be expected to produce yields in excess of 40t/ha in the high infestation found on this experiment site. However, to produce

differences in yield, irrigation was not used on this experiment, and fertiliser was not applied in an attempt to stress the crop in the hope that differences in yield could be produced if nematicide incorporation method really does have a significant effect on yield protection and PCN control. However, from this data it would seem that by withholding irrigation and nutrients the crop was too heavily stressed. This is highlighted by the low average total yield for the experiment of 19.7t/ha; under these artificially stressed conditions no significant differences in yield occurred.

Table 32. Yield by tuber size for Field Experiment 4

Incorporation method	<45mm (t/ha)	45-65mm (t/ha)	65+mm (t/ha)
Rotavator	2.1	11.7	3.9
Bed tiller	1.7	12.7	7.4
Bed tiller/destoner	2.1	10.0	6.9
Destoner	1.9	10.3	7.0
Untreated control	1.6	11.9	7.2
S.E. (4 D.F.)	0.17	0.87	1.19
C.V%.	26	22	52

Table 32 shows the tuber yields of each grade for Field Experiment 4. No significant differences occurred between treatments in any of the tuber grades. The withdrawal of irrigation and nutrients would seem to have affected the partitioning of tuber grades which is highlighted in the high percentage ware yields produced with a mean percentage ware of 89.91 and the main component of yield falling in tuber sizes >45mm.

5.4.3.3 Nematode population estimation

The results from PCN soil sampling after planting Field Experiment 4 showed little variation in Pi between incorporation treatments (Table 33). The mean Pi found for the Experiment of 79 eggs/g of soil is considerably higher than that found in the initial sampling, which showed a mean population density of 23 eggs/g of soil. The intention of this experiment was to use a low to moderate population in an attempt to produce high multiplication rates and thereby highlight any problems with incorporation techniques in terms of nematode control. Possible reasons for the anomaly between the initial sampling and the actual Pi's found on the experiment site are that the soil sampling for the Pi's was conducted on a much smaller area of 19m² compared with 400m² for the initial sampling. Another possible explanation for the difference between the initial sampling and the Pi's is that the initial soil samples for PCN were taken from unploughed soil, which had recently been cropped with red beet, whilst the Pi's were taken from deeply cultivated soil directly after planting. This highlights the limitations of soil sampling in a commercial situation, where it is not usually possible to take soil samples for PCN population estimation in soil conditions similar to those at potato planting. It may be prudent to conduct initial sampling for future field experiment sites on deeply ploughed land in order to obtain an accurate assessment of PCN populations present.

The final populations found in Field Experiment 4 (Pf) are also shown in Table 33. Significant differences ($P \leq 0.05$) were found between the untreated control and the stone and clod separator applying and incorporating Vydate. Incorporating Vydate with the stone and clod separator significantly reduced the increase in PCN, with a mean Pf of 119 eggs/g of soil compared with a mean Pf of 249 eggs/g of soil for untreated plots. No significant differences occurred between Pf values for nematicide incorporation treatments but all plots treated with nematicide showed

significantly lower ($P \leq 0.05$) multiplication (Pf/Pi) than the control. These results show that using a nematicide in this situation has reduced the multiplication of *G. pallida*, but do not show any significant effect of incorporation method on nematicide efficacy.

Table 33. Mean nematode populations (eggs/g of soil) pre planting and post harvest

Incorporation method	Pi* (eggs/g of soil)	Pf* (eggs/g of soil)	Pf/Pi*
Rotavator	78(4.335)	134(4.851)	1.71(0.517)
Bed tiller	80(4.328)	153(4.923)	1.93(0.597)
Bed tiller/destoner	78(4.287)	141(4.834)	1.85(0.549)
Destoner	77(4.193)	119(4.679)	1.78(0.486)
Untreated control	82(4.333)	249(5.433)	3.53(1.100)
S.E. (4 D.F.)	9.0(0.1264)	23.9(0.1358)	0.427(0.1511)
C.V%.	32(8)	42(8)	56(66)

*Natural log of data in parentheses

5.4.4 Conclusions

Overall, the data from Field Experiment 4 suggest that the method used to incorporate the granular nematicide Vydate is not as important as the fact that a nematicide is used. The method of incorporation had no effect on how well the nematicide performed. Crop growth, yield and nematode population changes remained unaffected by incorporation method, but were on the whole improved by the use of a nematicide, with crop growth increasing in terms of plant dry weights, and crop yield remaining relatively unaffected by the nematicide or lack of it. The populations of nematodes were affected by the nematicide with the untreated plots showing consistently higher Pf/Pi ratios than the nematicide treated plots. The method used to sample for PCN populations has been shown to be important in this particular experiment and suggestions have been made to improve it.

The use of a randomised block layout for Field Experiment 4, as opposed to a split plot design used in the other field experiments, has not given any further insight in terms of sensitivity to treatment variation. This experiment had greater replication than that used in other field experiments and this also has not shown any of the incorporation methods used to be unsatisfactory for nematicide incorporation.

Overall, the field studies using different nematicide incorporation methods have failed to show any significant differences between these treatments on crop growth, yield or PCN population dynamics.

6.0 CHAPTER 6

DETECTION AND QUANTIFICATION OF OXAMYL IN POTATO RIDGES OF FIELD EXPERIMENTS 1 AND 2

6.1 Availability, movement and transformation of granular nematicides

6.1.1 Introduction

The incorporation of granular nematicides into the soil is the first step in bringing a nematicide and nematodes into close proximity. The efficacy of soil applied nematicides after incorporation has taken place is then dependent on the exposure of nematodes to the nematicide in the soil solution. The availability of the nematicide in the soil solution after application and incorporation is dependent on several factors:

6.1.1.1 Adsorption

The granular nematicides aldicarb and oxamyl need to be part of the soil solution in order to affect PCN and the concentration of nematicide in the soil solution is critical for satisfactory PCN control. To assess the concentration likely to be achieved, a knowledge of the nematicide's adsorption onto soil organic matter is required. Soil adsorption of pesticides is commonly measured by the soil slurry technique (Bromilow *et al.*, 1980). The tendency of pesticides to become adsorbed onto soil organic matter is expressed as the coefficient of adsorption, which is defined as;

$$K_{om} = \frac{\text{amount adsorbed on organic matter (mg/Kg)}}{\text{concentration in soil solution (mg/dm}^3\text{)}}$$

The K_{om} values given in Table 34 show that oxamyl and aldicarb and its oxidation products are only weakly adsorbed onto organic matter. The two oxidation products of aldicarb are adsorbed more weakly than aldicarb itself. For oxamyl two different ranges of K_{om} values have been reported,

the reason for which may lie in the soil types used in the calculations of the values.

Table 34. K_{om} values of commercial nematicides and their oxidation products.

Compound	K_{om} (dm^3/ Kg)	References
Aldicarb	5 - 13	Bromilow (1973), Bromilow *et al.* (1980)
A. sulphoxide	0 - 4	Bromilow *et al.* (1980)
A. sulphone	2 - 6	Bromilow *et al.* (1980)
Carbofuran	17 - 37	Felsot and Wilson (1980)
Ethoprophos	43 - 68	Leistra (1979), Leistra & Smelt (1981)
Fenamiphos	100 - 141	Bromilow (1973), Lee *et al.* (1986)
F. sulphoxide	22	Lee *et al.* (1986)
F. sulphone	25	Lee *et al.* (1986)
Oxamyl	2 - 7	Bromilow (1973)
	12 - 45	Gerstl (1984)

From Smelt and Liestra 1992.

The adsorption of a nematicide by soil reduces the concentration in the soil solution and, consequently the bio-availability and the extent of movement with percolating water. However, aldicarb and oxamyl are generally not strongly adsorbed onto soil organic matter so their movement and availability should remain high and they can therefore be used successfully on a wide range of soil types.

6.1.1.2 Solubility in water

The solubility of a nematicide in soil water has implications for movement in the soil by leaching, and for availability of the nematicide. Nematicides such as oxamyl, which has a high solubility in water of 280g/l, and aldicarb, which has a solubility of 4.93g/l, are more likely to be leached in greater quantities by rainfall than are less water soluble compounds such as carbofuran which has a solubility of 0.32g/l (Anon, 1994). In terms of availability, the more water soluble nematicides will show greater efficacy in dry conditions than less soluble nematicides as more active ingredient is capable of dissolving in a smaller volume of water.

6.1.1.3 Movement

Weakly adsorbed nematicides such as oxamyl and aldicarb are more likely to be redistributed by soil water in a wide range of soil types than are more strongly adsorbed nematicides. Field studies in two loamy soils showed that ethoprophos (K_{om} 43-68) was leached deeper in soil with a low organic matter content. In the same studies, redistribution of ethoprophos by winter rainfall after incorporation was limited (Liestra and Smelt, 1981). Field studies in the spring using aldicarb showed a limited redistribution of its weakly adsorbed oxidation products (Smelt *et al.*, 1981b). However, no such studies have been conducted for oxamyl. The water infiltration of different soil types and soil conditions such as ridging

could have great effects on nematicide leaching. By ascertaining the exact placement of granules in the soil after potato planting the merits of each incorporation technique in relation to early plant growth could be achieved. Also by monitoring the position of a nematicide during the growing season, an understanding of the effects that soil water movement has on the re-distribution of the nematicide could be gained.

6.1.1.4 Transformation

Transformation of pesticides in the soil to non-toxic compounds is a natural process and is necessary to prevent the active compounds entering the food chain. In order to meet the requirements of registration the nematicides in use at present are of low to moderate persistency. Transformation rates are usually measured in incubation studies under controlled temperature and soil moisture conditions. Table 35 shows the transformation rates of several nematicides and their bio-active transformation products. Nematicides with lower transformation rates will provide longer protection for the crop roots but, in practice, the low dose rates used by growers will generally give only short lived reversible nematostatic action. The transformation rate of a nematicide is not static, it can vary between soil types and with soil conditions such as soil temperature and moisture. If repeated use of the same nematicide occurs over several seasons on the same ground, a phenomenon known as accelerated transformation can occur. Suett (1996) showed that one year after a single application of the commercially recommended dose of the granular formulation of carbofuran a second application was totally ineffective. Accelerated transformation of the nematicides by micro-organisms may be the cause. Smelt *et al.* (1987) studied the decreased control of PCN by aldicarb, ethoprophos and oxamyl in laboratory and field experiments. Measurements of nematicide persistence taken from

field plots treated two or five times previously, showed low persistence, and sterilisation of the treated soil led to a decrease of transformation rates, indicating that micro-organisms could be responsible for the accelerated transformation.

Table 35. Transformation rates of commercial nematicides.

Compound	Time for 50% transformation (days)	References
Aldicarb	2 - 9	Bromilow *et al.* (1980)
(total toxic residue)	18 - 90	Smelt *et al.* (1978a, 1978b, 1978c)
		Suett and Jukes (1988)
Carbofuran	20 - 167	Ahmad *et al.* (1979)
		Suett (1986)
Ethoprophos	16 - 120	Smelt *et al.* (1981a), (1987)
Fenamiphos	5 - 18	Lee *et al.* (1986)
Total toxic residue	70 - 190	Smelt *et al.* (unpublished)
Oxamyl	7 - 39	Bromilow (1973)
		Bromilow and Leistra (1980)
		Gerstl (1984)

From Smelt and Liestra, 1992.

6.2 The placement of oxamyl in potato ridges using a range of application and incorporation methods

6.2.1 Introduction

The distribution of granular nematicides after incorporation has been studied in great detail by Moss, Crump and Whitehead (1975) and Bromilow and Lord (1979) who used the rotavator, Dutch harrows and Roterras to incorporate granular nematicides into the soil prior to planting a potato crop. They found that the rotavator consistently and uniformly incorporated the nematicide to the working depth of the implement and gave better control of PCN as a result of this deeper, uniform incorporation. As described in Chapter 2, the equipment used for producing potato ridges is changing, and as a result the incorporation of granular nematicides may be less efficient than that of methods used in pre-bed potato production. This experiment aims to appraise the methods of incorporation currently in use and compare them with methods of incorporation used before the bed system of potato production. In addition, the influence of the potato planter is studied as this (from work done in Chapter 3) has potentially the greatest effect on nematicide distribution.

6.2.2 Materials and methods

6.2.2.1 Application and incorporation of nematicide

Oxamyl (Vydate 10% ww a.i. granules) was incorporated into the potato seed beds using the treatments described in Field Experiment 1 and Field Experiment 2 (Chapter 4). As the nematicide oxamyl was studied in this experiment, the methodology in Chapter 4 describing the nematicide aldicarb is not relevant.

6.2.2.2 Soil sampling

Three soil cores, 4cm diameter and 40cm deep, were taken, from plot positions chosen at random from either the first or second ridge of the centre treated ridges immediately after the potato crop was planted. Cores were taken at specific positions within the ridge (see Fig. 16). Each core was cut into 10cm lengths, and the individual samples stored at -16°C until analysis.

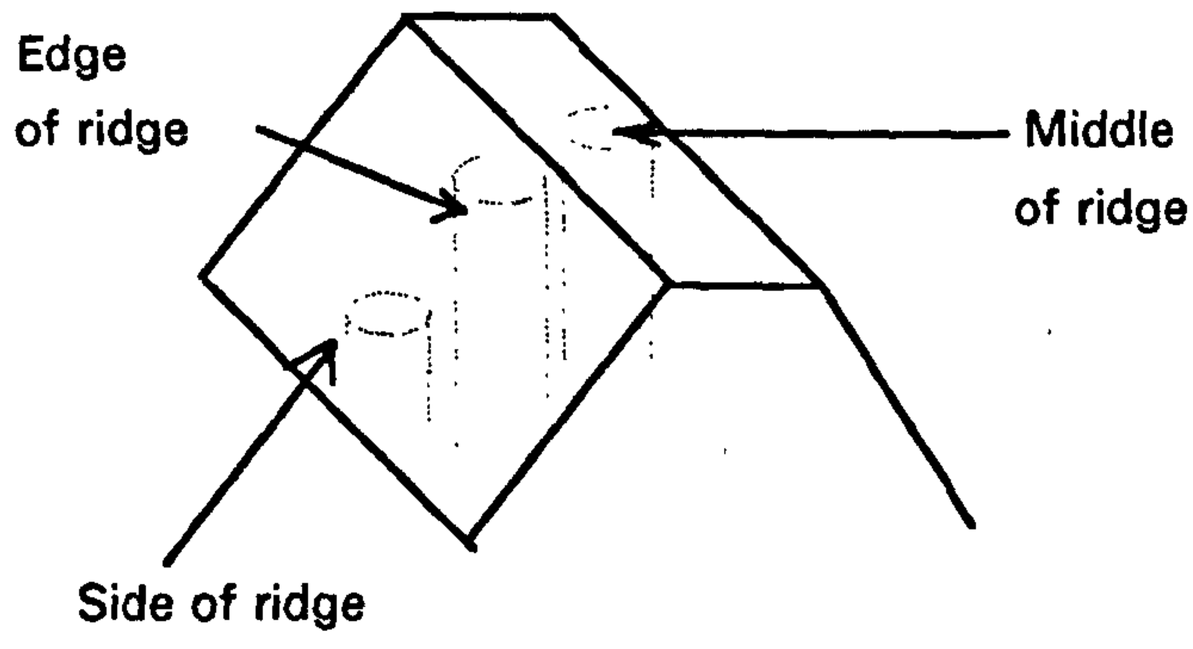


Figure 16. Diagram showing position of soil cores taken from a ridge

6.2.2.3 Chemical analysis

50g of moist soil containing oxamyl was shaken with 200ml of acetone for 4 hours on a reciprocating shaker. 20ml of the resulting supernatant was then taken to dryness using a rotary evaporator and the residue was dissolved with the help of an ultrasonic water bath, in 2ml of a 20% methanol / 80% distilled water solution. 1.5ml of the resulting solution was then placed in a centrifuge tube and the solution spun at 1800 RPM for 15 minutes to remove any soil particles. The centrifuged samples were then analysed by HPLC using an LDC Constametric model III and an LDC spectromonitor UV detector. A reverse phase 25 x 0.46cm i.d. cartridge column containing Sherisorb 50 DS protected by a 3 x 0.46cm i.d. guard column also containing Spherisorb 50 DS was used and a mobile phase

containing methanol / water (20 + 80 by volume). The eluant was monitored at 230nm with a retention time using a flow rate of 1.5 ml/min of 6 minutes (A. Evans *pers comm*). Analytical efficiency was determined by adding oxamyl solutions to untreated soil samples to give 0, 1 and 5µg / g of soil. Recoveries always exceeded 90%.

6.2.3 Results and discussion

The mean concentrations of oxamyl in µg/g dry soil for Field Experiment 1 are given in Tables 36 and 37. Results for Field Experiment 2 are given in Tables 38 and 39. Data obtained from the top 20cm is presented as only trace concentrations of oxamyl were found below 20cm for all the incorporation methods at both sites.

Overall, method of incorporation did affect the concentrations of oxamyl found in the top 20cm of the ridge for Field Experiment 1 (Table 36).

Table 36. The effect of incorporation method on oxamyl concentration (µg/g of soil) in the top 20cm of the potato ridge at planting for Field Experiment 1

Incorporation method	oxamyl concentration (µg/g soil)
Bed tiller followed by destoner	1.57
Bed tiller	4.25
Destoner	2.83
Vertical band applicator	3.30
S.E. (3 D.F.)	0.285
C.V.%	40.5

From Table 36 it can be seen that the bed tiller, destoner and vertical band applicator all placed significantly ($P \leq 0.01$) more oxamyl in the top 20cm of the ridge than the double incorporation method. Highest oxamyl concentrations were found in plots where the bed tiller had incorporated oxamyl. Concentrations were significantly ($P \leq 0.01$) higher for this treatment than the destoner and the vertical band applicator which both produced similar concentrations of oxamyl in the top 20cm of the ridge. These results agree with those found in Chapter 3 with two exceptions. The double incorporation method using a bed tiller followed by a destoner produced lower concentrations of oxamyl in the top 20cm of the row but, did not produce the deep distribution as seen in Chapter 3 as no significant concentrations of oxamyl were found below 20cm for this treatment. However, the concentration of oxamyl in the top 20cm of the ridge profile is lower than the anticipated concentration of 2.5µg/g of soil (Appendix 3). This may be due to dilution of the nematicide by the double incorporation but, the concentrations of oxamyl found below 20cm were negligible and showed little evidence that substantial amounts of nematicide have been incorporated deeply. These results therefore suggest that some nematicide has either been lost, or that the sampling method used was not adequate to detect oxamyl below 20cm.

The bed tiller applying and incorporating oxamyl produced the highest concentrations of oxamyl in the top 20cm of the ridge. The theoretical concentration of 2.5µg oxamyl/g of soil is exceeded for this treatment.. This might be explained by the action of the potato planter effectively folding treated soil together into bands that correspond with the depth sections of a soil core thereby increasing the amount of oxamyl present in the top 20cm. This is the most probable explanation as all machines were calibrated to apply the same rate of oxamyl (5.5kg a.i./ha) and regular

monitoring during experiment preparation avoided the same plots being treated twice by accident.

Table 37. Effect of incorporation method on the position of oxamyl concentrations (μg/g of soil) in the ridge at planting for Field Experiment 1

Incorporation method	Oxamyl concentration (μg/g soil) in ridge positions		
	side	edge	middle
Bed tiller followed by destoner	1.41	1.35	1.97
Bed tiller	2.60	4.87	5.28
Destoner	2.12	3.09	3.27
Vertical band applicator	4.49	2.69	2.70
S.E. (6 D.F.)	0.493		
C.V.%	40.5		

The position of oxamyl in the top 20cm of the ridge for incorporation methods used in Field Experiment 1 are shown in Table 37. From Table 37 the bed tiller placed significantly ($P \leq 0.001$) more nematicide in the edge and middle ridge positions than the other treatments. However, at the side of the ridge, the vertical band applicator placed a significantly higher concentration of oxamyl than the other treatments. The vertical band applicator was not originally designed to work on a potato bed system and had to be modified to suit this experiment (Chapter 2.). This may be the cause of the uneven distribution produced by this machine in this situation.

Table 38 shows the mean concentrations of oxamyl in the potato ridge for the incorporation treatments used in Field Experiment 2. Both the incorporation methods used in Field Experiment 2 produced concentrations of oxamyl in the top 20cm of the ridge close to the theoretical concentration of 2.5µg oxamyl/g of soil and would show benefits to crop growth in low to moderate PCN infestations.

The Pearson Megastar showed significantly ($P \leq 0.001$) higher concentrations of oxamyl in the top 20cm of the ridge than the rotavator. This may have been caused by experimental error such as overdosing the plots or, more likely, that the sampling method of using 10cm depth fractions may cause misleading results when an incorporation method causes concentrated bands of nematicide that correspond with a particular depth fraction. This is more likely to be the case here as from Chapter 3 it was shown that application of a nematicide halfway up a stone and clod separator produced concentrated bands of nematicide at the edges of the ridge.

Table 38. The effect of incorporation method on oxamyl concentration (µg/g of soil) in the top 20cm of the potato ridge at planting for Field Experiment 2

Incorporation method	oxamyl concentration (µg/g soil)
Pearson Megastar	3.72
Rotavator	1.89
S.E.(1D.F.)	0.338
C.V%	51

The Pearson Megastar does not use webs for stone separation, but the machine used in Field Experiment 2 did have the application equipment situated halfway up the bank of plastic stars.

Table 39 shows the position of oxamyl in the ridge after incorporation by the two treatments used in Field Experiment 2. Differences between incorporation technique and the position of oxamyl within the potato ridge occurred but were not found to be statistically significant.

The Pearson Megastar produced an uneven distribution of oxamyl, with highest oxamyl concentrations found in the side of the ridge. These results agree with those found in the fluorescent tracer granule work where application of tracer granules halfway up the first web of a stone and clod separator produced concentrated bands of tracer at the side of the planted bed.

Table 39. Effect of incorporation method on the position of oxamyl concentrations (µg/g of soil) in the ridge at planting for Field Experiment 2

Incorporation method	Oxamyl concentration (µg/g soil) in ridge positions		
	side	edge	middle
Pearson Megastar	4.49	2.90	3.77
Rotavator	1.49	2.08	2.10
S.E. (2 D.F.)	0.586		
C.V.%	51		

The concentrations of oxamyl found in the three ridge positions after oxamyl had been incorporated using a rotavator (Table 39) show that the side core contained less oxamyl than in the other two ridge positions. The

concentrations of oxamyl in the top 20cm of the ridge appears to be more uniformly distributed than those found after the Pearson Megastar treatment.

The rotavator used in Field Experiment 2 is almost identical in design to the bed tiller used in Field Experiment 1. Both machines produced even distributions of oxamyl in the middle and edge ridge positions, and the side ridge position showed the lowest concentrations of oxamyl for both these treatments. However, the bed tiller overall showed higher concentrations of oxamyl in Field Experiment 1 than those produced by the rotavator in Field Experiment 2. This difference in oxamyl concentrations between two similar machines needs further investigation.

6.2.4 Conclusions

The results presented show clearly that the application methods used all produce a distribution of oxamyl that is confined mainly to the top 20cm of the ridge, with no indications of oxamyl becoming over-diluted in the soil profile as little nematicide was found below 20cm. This is particularly important with reference to the stone and clod separator as Whitehead (1994b) suggested that a decrease in nematode control could be attributed to the use of these machines for incorporating granular nematicides and an over-dilution of the active ingredient in the soil. The method of initially incorporating oxamyl with a bed tiller followed by a stone separator showed less oxamyl in the ridge profile, which at first sight might be thought to be caused by the double incorporation diluting the product. However, little oxamyl was found below 20cm for this treatment, so unless the oxamyl was incorporated below 40cm it is difficult to explain the lower concentration of oxamyl in this treatment. This result appears to agree, at least in part, with the findings in Chapter 3, but no explanation is available for the lower than expected concentrations of oxamyl below 20cm for this treatment other than error in the sampling method used.

The incorporation of oxamyl by the bed tiller immediately before planting gave the highest concentrations of oxamyl in the ridge profile, approximately twice the expected value.

In terms of nematicide placement the treatments all seemed to be placing the bulk of the nematicide above the seed tuber as seed was planted at a depth of approximately 20-25cm. Potatoes are generally planted at this depth to reduce the risk of tuber greening, particularly in susceptible varieties such as Maris Piper. The middle ridge position showed the highest concentration of oxamyl for treatments except for the vertical band applicator and the Pearson Megastar. This ridge position will be closest to

the potato plant in its early stages of development and a high concentration of nematicide at this point will thereby provide protection from nematode invasion in the early stages of growth. However, more work is needed to find the optimum planting depth which places the seed tuber closest to the highest concentrations of a nematicide and also keeps tuber greening at an acceptable level. Smelt *et al.* (1981b) found in a similar experiment to this using aldicarb that highest concentrations of aldicarb and its metabolites were found in the area of the ridge where the potato roots developed. They also noted that, during the growing season, the amounts of nematicide were highest in the ridge and lowest in the furrow.

The effect of rainfall and irrigation on the movement of oxamyl later in the season may help to move the nematicide downwards further into the rooting zone. This will be studied in soil cores taken 3 weeks after planting.

Overall, this experiment leads to several conclusions regarding nematicide incorporation. If application of nematicide occurs at the front of the machine, the stone and clod separator is capable of incorporating granular nematicides uniformly without banding or diluting oxamyl in the ridge.

Incorporating granular nematicides with a bed tiller after stone and clod separation gives higher concentrations of oxamyl in the ridge profile after planting than the other methods used.

The incorporation of granular nematicides using a bed tiller followed by a stone and clod separator to give a second incorporation may give unsatisfactory results; however, further work is needed to deduce the fate of any "lost" nematicide.

The Pearson Megastar stone and clod separator produced an unsatisfactory, unevenly banded distribution of nematicide when application occurred halfway up the bank of stars used for stone separation.

The action of the potato planter may cause further incorporation and concentration of granular nematicides in the potato ridge.

The methods used for incorporating granular nematicide in this experiment all achieve a sufficient concentration of oxamyl in the early rooting zone of the crop after planting. Work described later in this Chapter will determine if movement of the nematicides later in the season provides sufficient concentrations of oxamyl deeper in the ridge (i.e. 20-40cm) to protect the roots as they work their way downwards.

6.3 The effect of incorporation method on the distribution of oxamyl in potato ridges three weeks after planting

6.3.1 Introduction

The placement of oxamyl in potato ridges has been studied and the results have been discussed earlier in this Chapter. The aim of this study was to determine the effect of incorporation method on the distribution of oxamyl later on in the season when other factors such as leaching by rainfall and irrigation and possible movement up the soil profile by evaporation have occurred. Also, the concentration of active ingredient available for controlling PCN later on in the season should give a useful indication of rate of transformation of the nematicide. Published work on the distribution of nematicides in the soil profile has concentrated on the initial distribution immediately after planting; information on the subsequent movement and transformation of oxamyl later in the growing season is unavailable or does not relate to field conditions or a growing potato crop.

6.3.2 Materials and methods

The materials and methods used in this experiment are identical to those used earlier in this Chapter. Soil cores for determination of soil oxamyl concentration were taken three weeks after planting on the 20th of May 1994. Details of rainfall and other climatic conditions are described later in this chapter.

6.3.3 Results and discussion

Tables 40-43 show the concentrations of oxamyl found three weeks after planting for Field Experiments 1 and 2. Negligible amounts of oxamyl (less than 0.5µg/ml) were found below 20cm in both experiments for all treatments indicating no redistribution of nematicide by leaching. No significant differences occurred between the 0-10cm and 10-20cm depth fractions for all treatments, therefore, mean data from the top 20cm is presented.

Table 40 shows the mean oxamyl concentrations found in the top 20cm three weeks after planting for Field Experiment 1. The concentrations of oxamyl are considerably lower than those found at planting, indicating that transformation of the active ingredient has occurred.

After three weeks the bed tiller and the destoner treatments both showed significantly ($P \leq 0.002$) higher concentrations of oxamyl in the top 20cm than the double incorporation method and the vertical band applicator. The concentration of oxamyl remaining after three weeks for the bed tiller and destoner treatments would cause nematode paralysis. However, at the lower concentrations obtained from the double incorporation and vertical band methods, little control of juveniles in the soil would occur as concentrations of oximecarbamates below 1µg/ml allow PCN juveniles to recover from their toxic effect (Hague and Pain, 1970).

The double incorporation treatment which was thought to produce an overly-deep nematicide distribution again showed no indications of the deep incorporation that occurred for this treatment in Chapter 3 as no significant amounts of oxamyl were detected below 20cm.

The bed tiller which produced significantly higher concentrations of oxamyl than the other treatments at planting showed similar oxamyl concentrations to those obtained by the destoner three weeks later. This

could be explained by a more rapid transformation of the higher oxamyl concentrations in the soil. This may be the case, but a more likely explanation assuming equal rates of nematicide transformation in all treatments, is a flaw in the sampling procedure adopted for this work

Table 40. The effect of incorporation method on oxamyl concentration (µg/g of soil) in the top 20cm of the potato ridge for Field Experiment 1 three weeks after planting

Incorporation method	oxamyl concentration (µg/g soil)
Bed tiller followed by destoner	0.43
Bed tiller	1.07
Destoner	1.36
Vertical band applicator	0.57
S.E. (3 D.F.)	0.182
C.V.%	89

Table 41 shows the mean concentrations of oxamyl for each of the three sampling positions after incorporation by the treatments used in Field Experiment 1. The variability of the data is so high that significant differences were undetectable. However, from Table 41 the position of oxamyl within the ridge for the bed tiller, double incorporation and destoner treatments are similar to those found at planting. No evidence for oxamyl movement laterally or vertically can be seen. The vertical band applicator treated plots however, showed a different oxamyl distribution three weeks after planting than seen at planting. Less oxamyl occurred in the side ridge position at three weeks than found in the other ridge

positions. This may be due to rainfall as the side ridge position is most likely to suffer from water erosion. Photo degradation of oxamyl may also have occurred at a higher rate in the side ridge position thereby further reducing the amounts of oxamyl present in vertical band treated plots.

Table 41. Effect of incorporation method on the position of oxamyl concentrations (µg/g of soil) in the top 20cm of ridge for Field Experiment 1 three weeks after planting

Incorporation method	Oxamyl concentration (µg/g soil) in ridge positions		
	side	edge	middle
Bed tiller followed by destoner	0.23	0.22	0.85
Bed tiller	0.79	0.87	1.57
Destoner	0.33	1.73	2.01
Vertical band applicator	0.12	1.00	0.61
S.E. (6 D.F.)	0.223		
C.V.%	89		

Table 42 shows the mean concentrations of oxamyl by depth for all treatments in Field Experiment 2 three weeks after planting. Both incorporation methods showed the highest concentrations of oxamyl in the top 20cm of the ridge with negligible amounts of oxamyl in the deeper sections. The Pearson Megastar again showed a significantly ($P \leq 0.003$) higher mean oxamyl concentration than the rotavator. Oxamyl concentrations in the top 20cm of ridge three weeks after incorporation by the Pearson Megastar were sufficient to give PCN control. However, from the more detailed results shown in Table 43 the higher concentrations found in plots incorporated by the Pearson Megastar remain unevenly

distributed and concentrated mainly at the side and edge of the ridge, which may not provide any useful control of PCN.

Table 42. The effect of incorporation method on oxamyl concentration (μg/g of soil) in the top 20cm of the potato ridge for Field Experiment 2 three weeks after planting

Incorporation method	oxamyl concentration (μg/g soil)
Pearson Megastar	1.22
Rotavator	0.58
S.E.(1D.F.)	0.129
C.V%	61

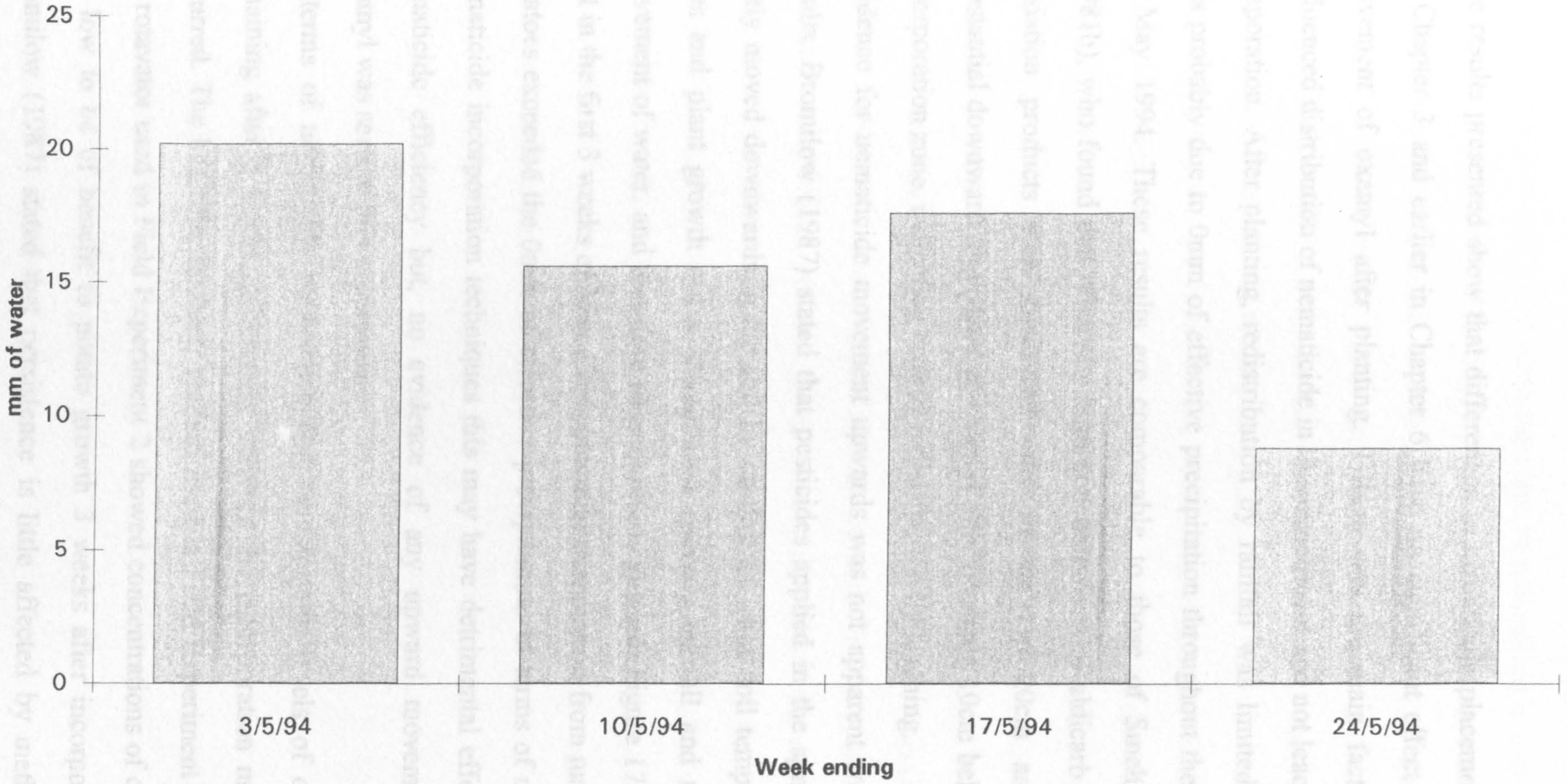
From Table 43 it can be seen that the Pearson Megastar has positioned oxamyl less uniformly than the rotavator. Significant differences between ridge positions seen immediately after planting were not significant three weeks later. However, the generally uneven nematicide distribution produced by the Pearson Megastar is still evident and whilst the average oxamyl concentration for the three ridge positions is sufficient to give useful nematode control, the position of oxamyl lying mainly at the top side and edge of the ridge is too far away from the growing potato roots to be of much benefit. The middle ridge position for Pearson Megastar incorporated plots is below the 1μg/ml threshold. This position is closest to the potato roots during the first three weeks of growth and therefore a low oxamyl concentration in the middle of the ridge but high at the outer edges indicates a potentially undesirable incorporation method.

The similarity in nematicide distributions for both treatments compared with those found at planting suggests that redistribution of oxamyl by leaching has not occurred.

Table 43. Effect of incorporation method on the position of oxamyl concentrations (μg/g of soil) in the ridge at planting for Field Experiment 2 three weeks after planting

Incorporation method	Oxamyl concentration (μg/g soil) in ridge positions		
	side	edge	middle
Pearson Megastar	1.44	1.25	0.98
Rotavator	0.58	0.52	0.64
S.E. (2 D.F.)	0.225		
C.V.%	61		

Fig. 17 Potential Evaporation (mm of water) for the first 3 weeks of May 1994 (MORECS data).



6.3.4 Conclusions

The results presented show that differences in nematicide placement seen in Chapter 3 and earlier in Chapter 6 have no significant effect on the movement of oxamyl after planting. Tillage was the main factor that influenced distribution of nematicide in this experiment and not leaching or evaporation. After planting, redistribution by rainfall was limited which was probably due to 0mm of effective precipitation throughout the month of May 1994. These results are comparable to those of Smelt *et al.* (1981b), who found that relatively high concentrations of aldicarb and its oxidation products were measured only in the top 20cm and that substantial downward movement of oxamyl only occurred 10cm below the incorporation zone, indicating limited redistribution by leaching.

Evidence for nematicide movement upwards was not apparent from the results. Bromilow (1987) stated that pesticides applied in the spring are rarely moved downwards in the soil by rainfall as, once soil temperature rises and plant growth starts, evaporation exceeds rainfall and the net movement of water, and therefore nematicide, is upward. Figure 17 shows that in the first 3 weeks of May 1994, potential evaporation from maincrop potatoes exceeded the 0mm of effective precipitation. In terms of modern nematicide incorporation techniques this may have detrimental effects on nematicide efficiency but, no evidence of any upward movement of oxamyl was seen in this experiment.

In terms of nematicide persistence and nematostatic levels of oxamyl remaining after 3 weeks, differences between the incorporation methods occurred. The bed tiller/destoner method used in Field Experiment 1, and the rotavator used in Field Experiment 2 showed concentrations of oxamyl too low to be of benefit to potato growth 3 weeks after incorporation. Bromilow (1987) stated that persistence is little affected by method of

application which may be correct in this situation, but an incorporation technique that produces lower concentrations of nematicide in soil solution initially will ultimately give concentrations of nematicide too low to provide much crop protection later in the growing season. The nematicide may have degraded at the same rate for all treatments but the levels persisting later in the season for the rotavator and double incorporation techniques are of no benefit to the potato plants.

Overall this experiment leads to the conclusion that three weeks after nematicide incorporation in low rainfall situations the initial distribution of nematicide in relation to the planted seed tuber is potentially the most important factor in nematicide efficacy. This may have been shown in the Field Experiments described in this thesis where no apparent biological effect on plant growth, tuber yield, root invasion or PCN control was observed between the incorporation treatments used. This is probably due to all the incorporation methods placing nematicide above the seed tuber. In the absence of downward nematicide movement to bring the developing roots into closer contact with the nematicide there is a reduction in the time that the potato roots are protected. Investigation of potato planting depth in relation to the depth of nematicide incorporation may well show improvements in nematicide efficacy.

7.0 CHAPTER 7

EVALUATION OF A DIAGNOSTIC ASSAY FOR THE DETECTION OF OXAMYL IN SOIL

7.1 Introduction

The pressure from consumers and environmental organisations to reduce the levels of agrochemicals that are applied to plants and their products emphasises the need for highly accurate and timely application of crop protection products. Furthermore, increasing competition in crop protection markets and the subsequent greater choice of products available to farmers means that existing products such as nematicides have to perform their task with greater efficacy. This situation is exacerbated by the intensive production methods for potatoes in the UK and a reliance on nematicide use on ground heavily infested with PCN. In addition certain production techniques such as incorporating granular nematicides with stone and clod separators (which from the previous chapters has been shown to be highly inefficient in some circumstances) may not allow nematicides to perform to their full potential. All these factors point to a need for assessing the effectiveness of nematicide incorporation techniques.

Individual nematicide granules are small (approximately 1-2mm in size) and not easily visible in the soil after incorporation making their distribution in a soil profile difficult to assess. The use of fluorescent tracer granules, as seen in Chapter 3, can help to identify problems with different incorporation methods. However, a method for rapidly assessing the concentration of oxamyl in the soil was required to detect problems as they occurred in the field. In response to requests for guidance from growers on how application machinery should be adjusted to minimise the efficacy problems caused by overdilution or banding of the product, DuPont (UK) Ltd has developed a diagnostic test called the Vytest to establish the depth to which oxamyl (Vydate 10G: 10% gr.) has been incorporated in the soil profile.

The aim of the experiments described in this chapter was to evaluate how well the Vytest detects oxamyl in the soil, and to assess whether complications are seen with the test results in the light of work undertaken in previous chapters.

7.1.1 The Vytest

The Vytest is a novel diagnostic kit used to detect oxamyl in soil. The field test is quick and simple to perform. After incorporation of oxamyl prior to planting, soil is sampled from potato beds to depths of 0 - 7.5cm (A) and 7.5 - 15cm (B) (Fig. 18). A 30ml subsample of soil is placed in a graduated tube with an equal volume of water. The soil / water mixture is shaken for 1 minute and allowed to settle for 3 minutes. The detector pad (Fig. 19) is placed in the slurry for 1 minute and then, after removing any adhering soil particles from the detector pad, it is developed by pressing the developer and detector pads together and holding them in place for 5 minutes. If the detector pad turns dark blue oxamyl is absent, a pale blue detector pad indicates that oxamyl is present in low concentrations and if the pad remains white then oxamyl is present in sufficient quantities to cause nematode paralysis (Anon, 1992).

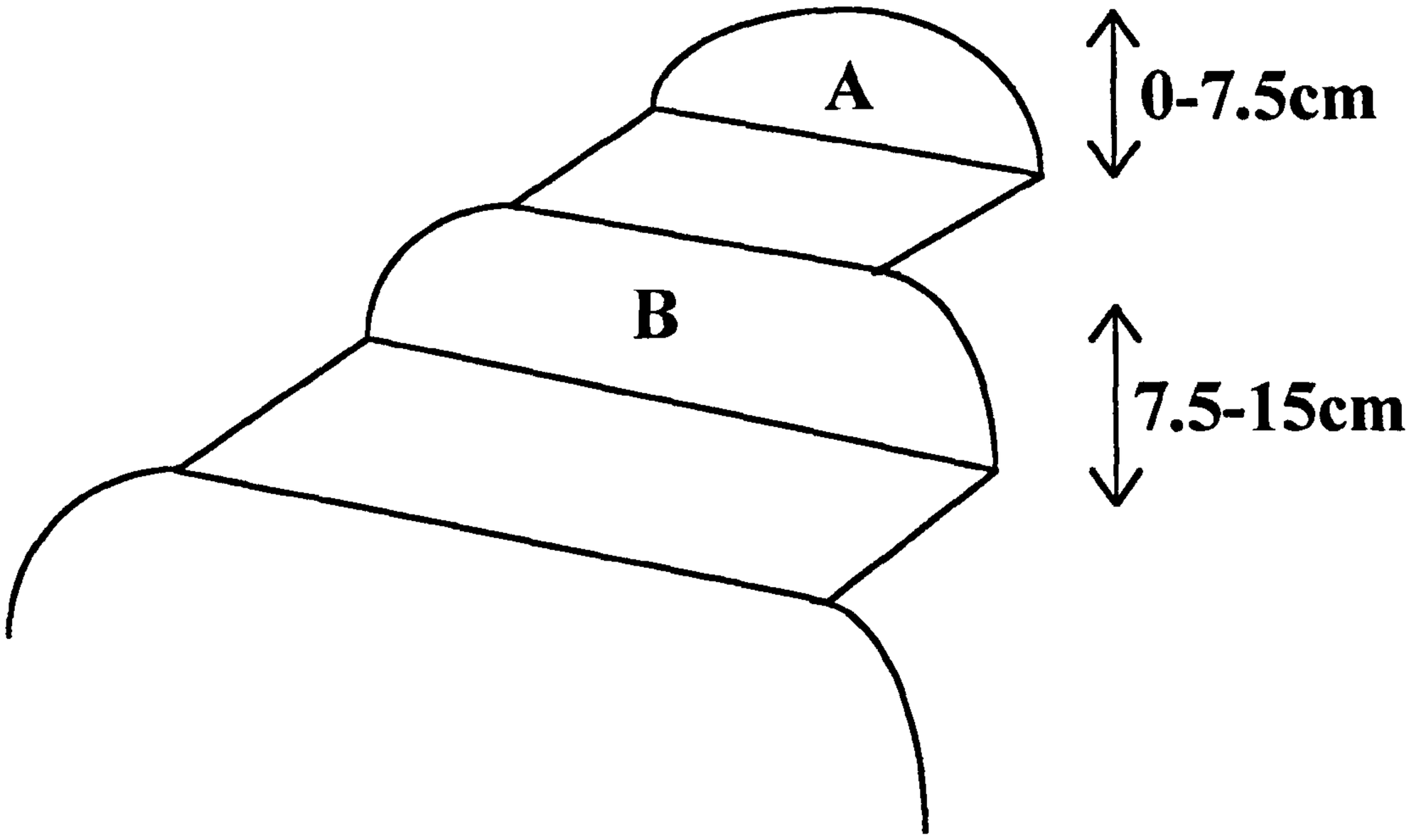


Fig. 18 Soil profile showing sampling depths

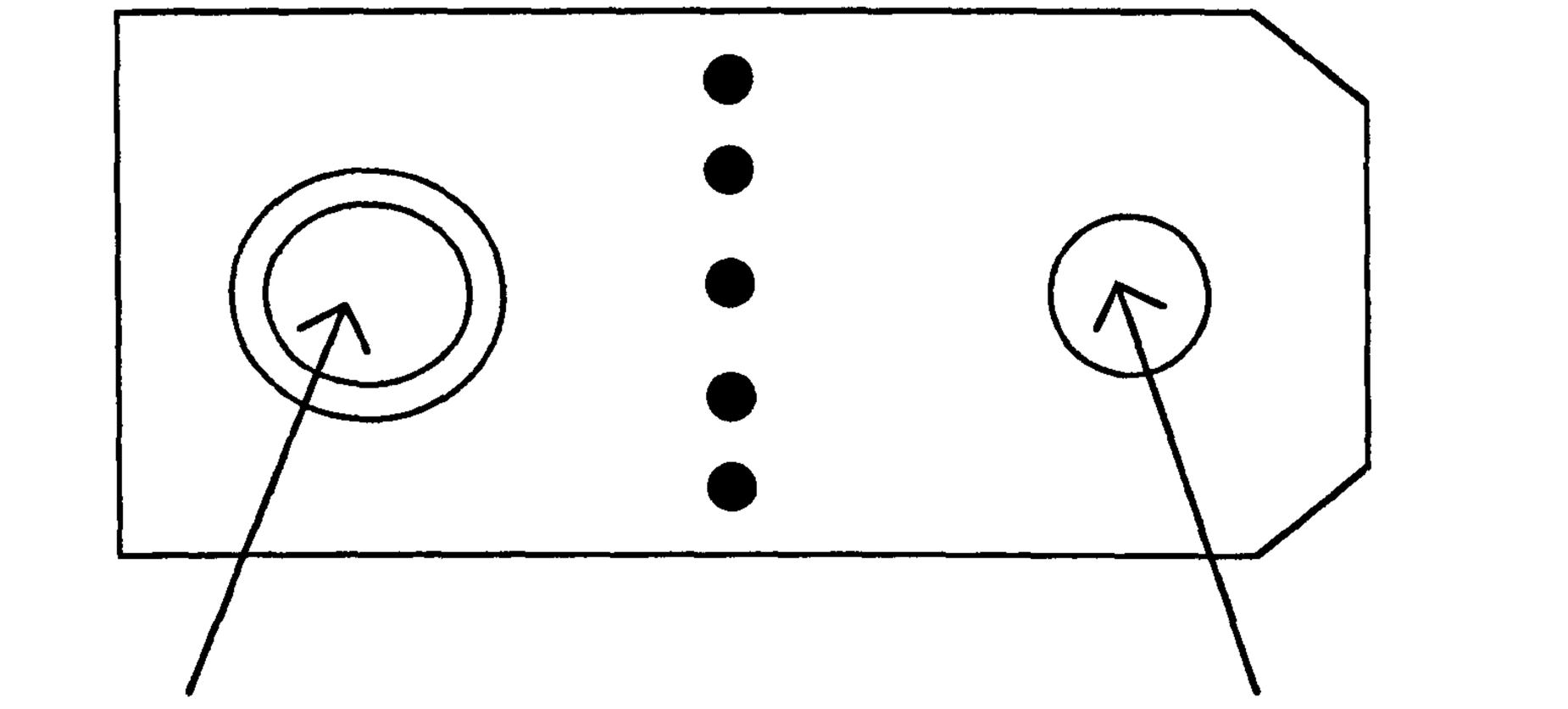


Fig. 19 Diagram showing the Vytest components

7.2 Materials and methods

7.2.1 Sensitivity of the Vytest to standard oxamyl solutions

Standard oxamyl solutions of 20, 10, 5, 4, 3, 2, 1, 0.5, 0.2, 0.1 and 0µg/ml were prepared in 80% distilled water and 20% methanol. An aliquot portion (250µl) was placed on the white detector pad of the Vytest and allowed to soak in for 1 minute, after which excess solution was removed by shaking the pad. The detector and developer pads were then pressed together and held in place using a paper clip. After incubation for 5 minutes at 25°C, the colour of the developer pad was observed. The test was repeated five times for each concentration.

7.2.2 Vytest sensitivity to soil samples containing known oxamyl concentrations

7.2.2.1 Soil samples

In 1994, from a field experiment on a sandy loam soil in Shropshire, soil cores of 4cm diameter and 40cm depth were taken randomly from oxamyl treated potato ridges immediately after a potato crop had been planted. Each core was cut into 10cm lengths, and the individual samples stored at -16°C until analysis was undertaken.

7.2.2.2 HPLC analysis

Soil samples were analysed using high pressure liquid chromatography, using standard methods developed at IACR - Rothamsted (A. Evans, *pers. comm.*).

7.2.2.3 Vytest analysis

A sample (10g) of thoroughly mixed soil containing a concentration of oxamyl previously determined by HPLC was weighed out into a 100ml glass shaking jar. Distilled water (10ml) was added to the soil and the resultant slurry was agitated on an orbital shaker at 200 rev/min for 3 minutes. Five Vytest detector pads were placed in the slurry for 1 minute, removed and washed with distilled water to remove any soil particles, and the developer and detector pads pressed together and secured with a paper clip. The tests were incubated at 25°C for five minutes after which the colour of the detector pad was observed.

7.3 Results and discussion

7.3.1 Sensitivity of the Vytest to standard oxamyl solutions

The Vytest was capable of detecting oxamyl in solution at concentrations as low as 1 µg/ml (Table 44). Concentrations of oximecarbamates above 1 µg/ml paralyse nematodes in the soil solution, preventing them from invading the host plant, and concentrations of 5 µg/ml kill PCN juveniles (Hague and Pain, 1970). The threshold for a positive result lies between 2µg/ml and 3µg/ml (Table 44). Whitehead *et al.* (1973b) suggested that oxamyl concentrations of 2.5µg/ml in soils planted with potatoes gave a satisfactory yield response in PCN infested soil and controlled PCN population increase compared with untreated controls. Therefore it would seem that the Vytest operates correctly, giving a positive result at oxamyl concentrations needed for satisfactory nematode control.

Table 44. The analysis of standard oxamyl solutions using the Vytest

	Concentration (µg/ml) of oxamyl										
Rep	20	10	5	4	3	2	1	0.5	0.2	0.1	0
1	W	W	W	W	W	W	PB	PB	DB	DB	DB
2	W	W	W	W	W	PB	PB	DB	DB	DB	DB
3	W	W	W	W	W	PB	PB	DB	DB	DB	DB
4	W	W	W	W	W	PB	PB	DB	DB	DB	DB
5	W	W	W	W	W	PB	PB	DB	DB	DB	DB

W=White detector pad, oxamyl present

PB=Pale blue detector pad, oxamyl present in low concentrations

DB=Dark blue detector pad, oxamyl absent

7.3.2 Sensitivity of the Vytest to soil samples containing known concentrations of oxamyl

The results from soil analysis by HPLC and the Vytest are shown in Table 45. The threshold between a positive and negative result for oxamyl detection by the Vytest lies at approximately 2µg/ml. The Vytest gave a positive result for 1.91µg/ml and a low concentration result for 2.26µg/ml, which may have been due to an uneven distribution of oxamyl in the sample used.

Table 45. The analysis of soil samples containing oxamyl using the Vytest and HPLC

Concentration of oxamyl using HPLC analysis (µg/g soil)	Vytest result Replicate				
	1	2	3	4	5
0	DB	DB	DB	DB	DB
1.07	PB	PB	PB	PB	PB
1.47	PB	PB	PB	PB	PB
1.91	W	W	W	W	W
2.26	PB	PB	PB	PB	PB
2.83	W	W	W	W	W
3.10	W	W	W	W	W
3.22	W	W	W	W	W
4.09	W	W	W	W	W
5.09	W	W	W	W	W

W=White detector pad, oxamyl present

PB=Pale blue detector pad, oxamyl present in low concentrations

DB=Dark blue detector pad, oxamyl absent

7.4 Conclusions

The threshold of detection for the diagnostic kit was well suited to the detection of concentrations of oxamyl expected in soil profiles following the correct incorporation of recommended rates of Vydate. Also, the results indicate that the Vytest is capable of detecting the presence of oxamyl in soil at concentrations that will give satisfactory nematode paralysis.

However, in some circumstances the test gave ambiguous results such as the positive result at 1.91µg/ml and a low concentration result at 2.26µg/ml. In practice, failure to detect oxamyl in the field is generally followed by resampling and double checking in case of sample variation. If several samples from soil depths of 0-7.5cm and 7.5-15cm show oxamyl in low concentrations, then the incorporation technique used should be checked for any problems.

A potential disadvantage of the Vytest routine is that soil sampling occurs before planting. Sampling is necessary at this stage so that potential problems are rectified before a large area has been treated. Results discussed in Chapters 3 and 6 show that the action of a potato planter has an effect on the distribution of nematicide in the soil profile and therefore it would be prudent for soil sampling to take place after planting has occurred.

Diagnostic kits need to be calibrated so that, when giving qualitative information, the sensitivity of the test is such that "false positives" are avoided. The concentration in a soil sample must be reflected in the concentration of oxamyl in the test slurry to avoid such false positive results. Assuming that the water content of the soil is negligible and that all the oxamyl dissolves in the water within 3 minutes, the test should give a reasonable indication of oxamyl concentration in a sample. As the extraction is done on a 1:1 volume basis in the field, this should provide accurate results regardless of soil type, as granular nematicides are incorporated into a defined volume rather than a particular mass of soil. The evaluation of the Vytest indicates that, if used correctly, such false readings should not occur.

The use of diagnostic kits to improve the efficacy of agrochemical application will become increasingly important as efforts are made to minimise product wastage and environmental contamination. The

implications that the Vytest has for nematicide use are important in ensuring that granular nematicides are used accurately, efficiently and responsibly.

Three years of field experience with the diagnostic kit has demonstrated the advantage of being able to provide growers with guidance on incorporating Vydate (T. Dawkins *pers. comm.*). A number of cases have shown that Vydate has been banded in the soil, or diluted to such an extent that detection was not achieved. Adjustment of the cultivation process remedied the situation, thereby preventing potential under-performance of the product which inevitably leads to customer dissatisfaction.

However, the very nature of granular nematicide application and incorporation means that it is susceptible to mistakes and poor results. The application equipment is usually cheap but, generally, not user friendly. The number of moving parts open to wear and tear is high, and the difficulty in changing between products can lead to incorrect application rates. For example, aldicarb and oxamyl are applied at different rates and this can lead to over or under application of the products if they are changed without re calibrating the application equipment. Nematicide use in potato production is costly, but the benefits that its use can bring in protecting the yield of the crop and in ensuring that nematode population levels are controlled at a reasonable level for future potato production are very valuable. However, without correct and thorough calibration of application equipment, a nematicide is unlikely to achieve optimal results. In this respect, diagnostic kits such as the Vytest have an important role to play in ensuring that agrochemicals are not misused.

8.0 CHAPTER 8

GENERAL DISCUSSION, CONCLUSIONS AND FUTURE WORK

8.1 General discussion and conclusions

The aims of the research undertaken were to study the distribution of granular nematicides after incorporation by a range of traditional and modern potato bed cultivation practices. This information was then related to the growth and yield of potato crops and the population dynamics of PCN. The potential for making improvements to nematicide placement when using the Vytest diagnostic kit was also evaluated.

Pests and diseases that are distributed throughout the rooting zone are difficult to control (Bromilow, 1987). This is certainly the case for PCN and in particular *G. pallida*. As discussed in Chapter 1 the range of control measures available, when integrated, can make potato production on PCN infested ground economic. However, populations of *G. pallida* present in many of the UK's ware growing area are not controlled as well as they could be. As no fully *G. pallida* resistant potato cultivar or fully effective granular nematicide is available, growers should recognise that crop rotation is still one of the best options available to combat this costly pest of the potato crop.

From Chapter 2 it is evident that the range of cultivation practices used for incorporating existing granular nematicides in potato beds is cause for concern. No single method (apart from broadcasting the granules on flat ground followed by rotavation) is fully recommended by manufacturers, and the use of potato beds and stone and clod separation often renders this recommendation impractical.

With the limited time and resources available it was decided to study the initial distribution of a granular nematicide in the soil after incorporation by traditional and modern cultivation machinery. The results from the initial placement work described in Chapter 3 showed dramatic differences between some of the incorporation techniques. The double incorporation

method using a bed tiller followed by a stone and clod separator gave a very deep and thorough incorporation of tracer granules. This technique is used by growers who are uncertain of the incorporation capabilities of stone and clod separator alone but was identified as a potentially inefficient method of nematicide incorporation. The second incorporation method identified as potentially inefficient was the use of a stone and clod separator with nematicide applied half way up the first separation web. This technique produced a concentrated band of tracer granules in the top edges of the planted ridge and had previously been identified as potentially problematic by nematicide manufacturers. It has now been abandoned by most growers.

Once the incorporation characteristics of the various techniques had been assessed, the biological implications were investigated in Field Experiments 1 to 4 (Chapters 4 and 5). As stated by Wheatley (1977), biological effectiveness is frequently not very sensitive to the method of applying chemicals. This became apparent in the results from these experiments, with no effect on potato growth or yield and no effect on PCN populations occurring due to any particular incorporation method. Many reasons were discussed for the similarity in yield protection and PCN control exhibited by the incorporation methods. The choice of potato cultivars for the field experiments may have been inappropriate. The use of a partially resistant cultivar such as Santé in Field Experiment 1, and the use of tolerant varieties such as Fianna, Cara and Maris Piper may have made it difficult to show differences between incorporation methods in terms of PCN control or yield protection. Cultivars with great intolerance and low resistance to PCN infestation, such as Pentland Dell, could have been grown but the information would not have been useful to many growers as at the start of this research the area of Pentland Dell grown was small and getting smaller. Also, information produced on one cultivar may

not be representative for all cultivars. This research could provide important information to growers in the future if a wider range of tolerant, susceptible and resistant cultivars were used, as the agronomy of the potato crop is becoming increasingly specialised. It may be that one incorporation method is suitable for all crops and husbandry practices but, on the other hand, it may be that particular cultivars of certain tolerance or resistance benefit from nematicide incorporation done differently to that for a susceptible cultivar.

Chapter 6 was aimed at providing evidence for any biological differences that occurred in Field Experiments 1 and 2. No significant differences occurred between incorporation methods in the field experiments but, from the HPLC analysis of soil samples taken from these trials it became clear that some of the incorporation methods produced potentially undesirable distributions of oxamyl. However, these differences did not show up in crop growth or PCN control which further highlights the unfortunate choice of cultivar in the field experiments or leads to the conclusion that nematicide distribution is less important than the fact that a nematicide is used in the first place.

The efficacy of oxamyl when formulated as granules is determined by the toxicity of the active ingredient and also by the degree of contact that the active ingredient makes with PCN. The contact of nematicide and nematode is determined by the initial distribution of the nematicide in the soil, the rate of release of the active ingredient, the redistribution of the nematicide in the soil by leaching and the extent to which PCN moves in the soil matrix (Bromilow, 1987). The fact that all potato crops were planted below the nematicide treated layer in the potato ridge and consequently the developing root system had less time in contact with the nematicide was concluded to be responsible for the similarity in results gained from the different incorporation methods used in Field Experiments

1 to 4. In the first three weeks after planting when nematicide concentrations are generally highest, the majority of developing roots are concentrated around the base of the shoots close to the seed tuber. These roots are unlikely to be protected by nematicide if planting has been below the nematicide treated layer. Improvements to nematicide efficacy might be gained by bringing the early root system into more direct contact with nematicide. This could be achieved by either planting more shallowly and risk tuber greening, or by incorporating nematicide in such a way that the circular concentration of nematicide as described in Chapter 3 is produced deeper in the ridge. If this could be achieved leaving the top 5cm of ridge untreated noticeable improvements in efficacy could occur.

Chapter 7 aimed to evaluate a diagnostic kit called the Vytest which has been developed by DuPont in order to assist the efficient incorporation of Vydate by growers. This diagnostic kit was concluded to be a useful tool in improving the accuracy of nematicide placement. The Vytest is probably responsible for the abandonment of applying granular nematicide halfway up a stone and clod separator web, and several other inefficient incorporation practices. However, many growers have been recommended to stop using stone and clod separators for nematicide incorporation and have subsequently adopted the double incorporation method, which has been shown to provide over-deep incorporation of oxamyl and, 3 weeks after planting, showed very low non-toxic levels of oxamyl which would presumably give little benefit to the potato crop.

In terms of nematicide incorporation method and the placement of oxamyl in the planted potato bed, the double incorporation method of bed tiller followed by stone and clod separation is cause for concern. However, the acceptable nematicide distributions produced by the majority of cultivation practices may when planting is deep (greater than 20cm), miss the roots which are most vulnerable to PCN invasion. The following section on

further research aims to provide some ideas that may help to solve this problem and allow granular nematicides to perform to their potential.

8.2 Areas for future research

8.2.1 Irrigation

Potato root systems are likely to grow faster in spring than nematicide movement by natural rainfall. Therefore, enhancing movement by irrigation immediately after planting may provide scope for improving nematicide efficacy in a dry spring. Irrigating at different time intervals after planting may also help to move nematicde down around potato cultivars susceptible to greening that need to be deeply planted. Contamination of ground water by several pesticides, including aldicarb metabolites, has been observed in areas of the USA (Garner *et al.*, 1986), this leaching problem often having been exacerbated by heavy irrigation in treated fields. Therefore, application of different volumes of water in relation to soil moisture deficit could also be investigated in the first 3 weeks after planting, together with soil monitoring for nematicide distribution in order to prevent contamination of ground water.

8.2.2 Cultivar

More work is needed on the interaction of potato cultivars and incorporation method to provide for example, knowledge on how a susceptible, low tolerance cultivar performs under different nematicide incorporation techniques. More information on the variation of cultivar root distributions in the first three weeks after planting is needed along with knowledge of how planting depth and tuber greening are related.

8.2.3 Granular nematicide formulation

Research looking at different formulations of nematicides, i.e. liquids or different granule formulations (such as slow release), may show benefits over conventionally formulated nematicides. The distribution of chemical

achieved by the incorporation methods described in this thesis may limit nematode control, so could we expect to get better results by using more granules i.e. smaller or more lightly loaded granules? Different incorporation methods may work more efficiently with different granule formulations. For instance, the double incorporation of bed tiller followed by destoner might work better if the granules are formulated at 5% a.i and the application rate doubled.

8.2.4 Wind

The effect of high winds on incorporation during destoning should be investigated. Nematicide granules at application are dry and light in relation to the bulk of soil passing through a destoner. A winnowing effect has been noted during the implementation of Field Experiments with nematicide granules blowing sideways from underneath the separating web before they reach the soil surface. A fluorescence method of monitoring spray drift could be adapted to investigate this potential problem; tracer granules could be caught on sticky poles placed downwind of a stone and clod separator and any residues on the sticks could then be analysed.

8.2.5 Root invasion parameters

Knowledge of how root invasion progresses according to temperature, potato cultivar, soil moisture and nematicide for a range of sites throughout the country is unavailable. Gaining such information would be expensive and time consuming but, over a few years it would give a good idea of how invasion occurs over time and according to temperature, moisture and nematicide in actual field conditions. This basic knowledge could be used to help design efficient nematicide application practices tailor-made for a range of conditions.

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